

Science

25 October 2013 | \$10

AAAS ANNUAL MEETING

MEETING GLOBAL CHALLENGES:
DISCOVERY AND INNOVATION

13–17 FEBRUARY 2014
CHICAGO



EDITORIAL

- 402 Bricks and MOOCs
Marcia McNutt

NEWS OF THE WEEK

- 406 A roundup of the week's top stories

NEWS & ANALYSIS

- 409 Ancient DNA Links Native Americans With Europe
>> Science Podcast
- 410 U.S. Shutdown Ends, but Not Budget Anxiety
- 411 Earliest Known Galaxy Formed Stars at a Breakneck Pace
- 412 U.K. Science Adviser Faces Emotionally Charged Issues and Uncertain Science
- 413 Immune Suppressant Unexpectedly Boosts Flu Vaccine
- 415 Surprising New Dengue Virus Throws a Spanner in Disease Control Efforts

NEWS FOCUS

- 416 Varmus's Second Act
A Cancer To-Do List

LETTERS

- 420 Ecosystem Services: Accounting Standards
C. Obst et al.
- Ecosystem Services: The Farmers' Challenge
A. Graham et al.
- Ecosystem Services: Nature's Balance Sheet
R. Aspinall and P. Gregory
- Response
I. J. Bateman et al.

BOOKS ET AL.

- 423 Life Atomic
A. N. H. Creager, reviewed by J. Radin
- 424 Invasive Species
D. Simberloff, reviewed by M. Vilà

POLICY FORUM

- 425 Biodiversity Risks from Fossil Fuel Extraction
N. Butt et al.

PERSPECTIVES

- 427 Women, Fertility, and the Rise of Modern Capitalism
A. Alesina
- 428 Natural Selection and Pain Meet at a Sodium Channel
G. R. Lewin
>> Research Article p. 441
- 429 Expanding the Scope of Fluorine Tags for PET Imaging
L. Zhu et al.
- 431 The New Core Paradox
P. Olson
- 432 Mucus Coat, a Dress Code for Tolerance
Y. Belkaid and J. Grainger
>> Research Article p. 447
- 433 Blooms Bite the Hand That Feeds Them
H. W. Paerl and T. G. Otten

SCIENCE PRIZE ESSAY

- 436 Radiation and Atomic Literacy for Nonscientists
A. Johnson

CONTENTS continued >>



page 416



page 424

ON THE WEB THIS WEEK

>> Science Podcast

Listen to stories on shaping liquids with nanoparticles, eating scorpions with ease, the first Americans, and more.

>> Find More Online

Check out *Science Express*, our podcast, videos, daily news, our research journals, and *Science Careers* at www.sciencemag.org.



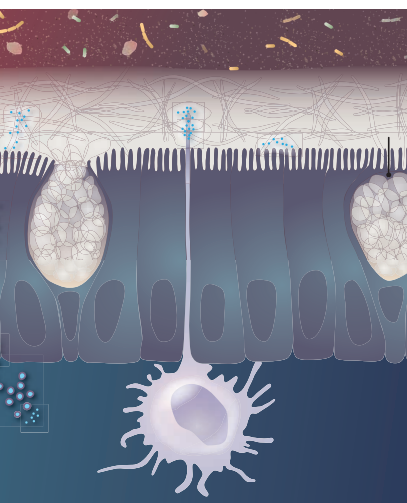
COVER

The 2014 AAAS Annual Meeting will take place 13 to 17 February in Chicago, Illinois. The meeting's theme—*Meeting Global Challenges: Discovery and Innovation*—focuses on finding sustainable solutions through inclusive, international, and interdisciplinary efforts that are most useful to society and enhance economic growth. The preliminary program begins on page 486.

Images (clockwise from top left; all ©iStock.com): pawel.gaul, deliormanli, Sean_Warren, ekvals, JamesReillyWilson

DEPARTMENTS

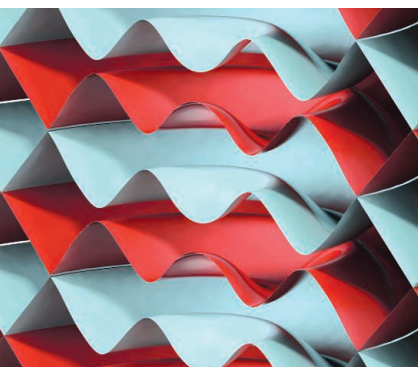
- 401 This Week in *Science*
- 403 Editors' Choice
- 404 *Science* Staff
- 438 AAAS News & Notes
- 485 New Products
- 486 AAAS Meeting Program
- 496 *Science Careers*



pages 432 & 447



pages 428 & 441



page 453

RESEARCH ARTICLES

- 440** Fine Tuning of Craniofacial Morphology by Distant-Acting Enhancers
C. Attanasio et al.
Targeted deletion of individual craniofacial enhancers from the mouse genome sculpts facial shapes.
Research Article Summary; for full text:
<http://dx.doi.org/10.1126/science.1241006>
>> *Video*
- 441** Voltage-Gated Sodium Channel in Grasshopper Mice Defends Against Bark Scorpion Toxin
A. H. Rowe et al.
Grasshopper mice use the scorpion's strength against it—instead of inducing pain, scorpion venom blocks pain.
>> *Perspective p. 428; Science Podcast; Video*
- 447** Mucus Enhances Gut Homeostasis and Oral Tolerance by Delivering Immunoregulatory Signals
M. Shan et al.
Mucus not only forms a physical barrier in the intestine but also promotes immunological tolerance of bacteria and foods.
>> *Perspective p. 432*

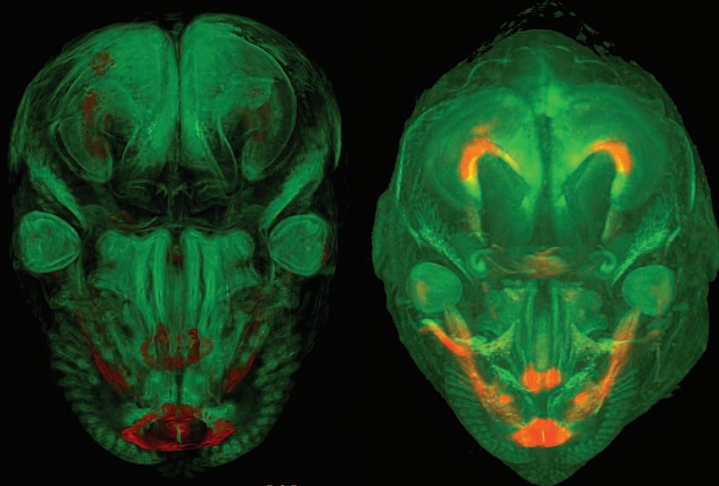
REPORTS

- 453** Observation of Floquet-Bloch States on the Surface of a Topological Insulator
Y. H. Wang et al.
Time- and angle-resolved photoemission spectroscopy is used to observe coherent coupling of light with electronic states.
- 457** From Few to Many: Observing the Formation of a Fermi Sea One Atom at a Time
A. N. Wenz et al.
Interactions in a one-dimensional few-atom gas of ^6Li exhibit a fast convergence to the limit of many particles.
- 460** Stabilizing Liquid Drops in Nonequilibrium Shapes by the Interfacial Jamming of Nanoparticles
M. Cui et al.
A self-assembling composite surfactant can be used to make stable droplets with complex shapes.
>> *Science Podcast*

- 463** Mass-Independent Oxygen Isotopic Partitioning During Gas-Phase SiO_2 Formation
S. Chakraborty et al.
The earliest solids in the solar system may have formed from a gas-phase reaction of SiO and OH .
- 466** Strong Premelting Effect in the Elastic Properties of hcp-Fe Under Inner-Core Conditions
B. Martorell et al.
Elastic weakening of iron just before melting explains variations in the seismic structure of Earth's inner core.
- 468** Atypical Combinations and Scientific Impact
B. Uzzi et al.
Highly cited work is simultaneously conventional and unconventional.
- 472** A Radical Intermediate in Tyrosine Scission to the CO and CN^- Ligands of FeFe Hydrogenase
J. M. Kuchenreuther et al.
Electron paramagnetic resonance spectroscopy elucidates a key step in the biosynthesis of hydrogenase active site ligands.
- 475** Causes and Effects of N-Terminal Codon Bias in Bacterial Genes
D. B. Goodman et al.
The structure of the 5' ends of messenger RNAs downstream of the start of protein translation has a major effect on protein expression.
- 479** 2000 Years of Parallel Societies in Stone Age Central Europe
R. Bollongino et al.
Genetic and isotopic evidence document changes occurring in Europe during the Neolithic era.
- 482** Changing Social Norm Compliance with Noninvasive Brain Stimulation
C. C. Ruff et al.
Right lateral prefrontal cortex activity affects sanction-based compliance with behavioral norms in humans.

SCIENCE (ISSN 0036-8075) is published weekly on Friday, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue, NW, Washington, DC 20005. Periodicals Mail postage (publication No. 484460) paid at Washington, DC, and additional mailing offices. Copyright © 2013 by the American Association for the Advancement of Science. The title SCIENCE is a registered trademark of the AAAS. Domestic individual membership and subscription (51 issues): \$149 (\$74 allocated to subscription). Domestic institutional subscription (51 issues): \$990; Foreign postage extra: Mexico, Caribbean (surface mail) \$55; other countries (air assist delivery) \$85. First class, airmail, student, and emeritus rates on request. Canadian rates with GST available upon request, GST #1254 88122. Publications Mail Agreement Number 1069624. Printed in the U.S.A.

Change of address: Allow 4 weeks, giving old and new addresses and 8-digit account number. Postmaster: Send change of address to AAAS, P.O. Box 96178, Washington, DC 20090-6178. Single-copy sales: \$10.00 current issue, \$15.00 back issue prepaid includes surface postage; bulk rates on request. Authorization to photocopy material for internal or personal use under circumstances not falling within the fair use provisions of the Copyright Act is granted by AAAS to libraries and other users registered with the Copyright Clearance Center (CCC) Transactional Reporting Service, provided that \$30.00 per article is paid directly to CCC, 222 Rosewood Drive, Danvers, MA 01923. The identification code for Science is 0036-8075. Science is indexed in the Reader's Guide to Periodical Literature and in several specialized indexes.



No Two Faces Are Alike

Gene disruptions can cause severe dysmorphologies like cleft palate, but what causes the subtle shifts in facial morphology that make each face unique? Studying mice, **Attanasio *et al.*** (p. 440) identified over 4000 candidate genetic enhancers around genes driving craniofacial development. To avoid the challenge of recognizing individual mouse faces, optical projection tomography was used to link changes in facial morphology with alterations in the function of specific enhancers.

Bite Me!

As unpleasant as it is, pain serves a purpose, to alert the body to potential damage. This protective function may explain why few predators have evolved resistance to the painful venoms used as a defense by their prey. The grasshopper mouse, however, is insensitive to one of the most painful stings in the animal kingdom—that of the bark scorpion. **Rowe *et al.*** (p. 441; see the Perspective by **Lewin**) now show that grasshopper mice use the toxins present in the scorpion venom to block voltage-gated pain transmission, temporarily reducing their sensitivity to nonvenom-induced pain. Thus, grasshopper mice use the scorpion's painful defense to their advantage and have evolved a mechanism that allows for reduction of pain sensitivity only when it is needed.

On the Brink of Melting

The considerable pressures and temperatures of Earth's iron-rich inner core make it a challenge to compare measurements made in experimental systems with observed seismic data. Computational simulations of core materials may reconcile any apparent differences. **Martorell *et al.*** (p. 466, published online 10 October) used ab initio simulations to predict the elastic properties of iron at core pressures. As temperatures approached the melting point of pure iron, the material was predicted to weaken to the point that seismic waves would be slowed considerably. An inner core with a small percentage of light

elements like oxygen and silicon near its melting temperature would correspond well with measured seismic velocities.

Not Very Many

In physics, the behavior of a system sometimes becomes easier to grasp when the number of particles is large and statistics begin to matter, but knowing how large the system needs to be for that to happen is a challenging computational problem. **Wenz *et al.*** (p. 457) used a one-dimensional trapped gas of ^6Li atoms to study this crossover from few to many. To simplify the problem, they worked with one "impurity" atom that was in a state unlike the other—"majority"—atoms. For weak and intermediate interactions, the system approached the many-body limit with as few as four majority atoms.

Explaining Anomalous Early Isotopes

Meteorites contain some of the oldest materials formed in the solar system, including silicate minerals similar in composition to those on Earth. These meteorites, however, often differ in their oxygen isotopic composition, implying that their formation involved a different chemical mechanism than younger solar-system materials. **Chakraborty *et al.*** (p. 463) performed a series of experiments and found that mixing of SiO gas and OH leads to a mass-independent

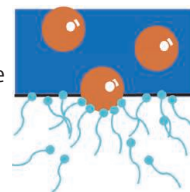
isotope fractionation in SiO_2 solid products similar to those observed in the oldest meteorites. This oxidation reaction may thus have served as the first source for silicates with anomalous oxygen isotopic compositions in the solar nebula.

Making an Impact

How big a role do unconventional combinations of existing knowledge play in the impact of a scientific paper? To examine this question, **Uzzi *et al.*** (p. 468) studied 17.9 million research articles across five decades of the Web of Science, the largest repository of scientific research. Scientific work typically appeared to draw on highly conventional, familiar mixtures of knowledge. The highest-impact papers were not the ones that had the greatest novelty, but had a combination of novelty and otherwise conventional combinations of prior work.

Dynamic Surfactants

Surfactants are used to form a stable interface between two nonmiscible liquids, like oil and water, so that droplets of one fluid can be entrained in the other. **Cui *et al.*** (p. 460) designed a surfactant based on the association of a hydrophilic nanoparticle with a functionalized oleophilic molecule that self-assembles at a water-oil interface to produce a composite surfactant. Once adsorbed, the nanoparticles tend to remain in place causing them to accumulate and "jam" at the interface. When a drop was deformed, more surfactant could assemble at the surface, allowing droplets of various shapes to be produced.



Conform to the Norm

Human societies have always enforced compliance with norms of acceptable behavior among their members by threatening punishment. It has been proposed that the human brain may have developed neural processes that support norm enforcement behavior and generate appropriate behavioral responses to social punishment threats. However, evidence for the neural circuitry underlying sanction-induced norm compliance in humans is limited. Using noninvasive brain stimulation, **Ruff *et al.*** (p. 482, published online 10 October) observed that alteration of the activity and excitability of the right lateral prefrontal cortex affected norm compliance, without affecting awareness of the content of the respective norms, or the expected sanctions. These alterations were much larger in a social, as compared to a nonsocial, context.

Additional summaries

Guardian of the Gut

The intestine is able to tolerate continual exposure to large amounts of commensal bacteria and foreign food antigens without triggering an inappropriate inflammatory immune response. In the large intestine, this immunological tolerance is thought to occur via a physical separation between environment and host imposed by a continuous mucous layer built up from the secreted mucin protein, MUC2. However, in the small intestine, this mucous layer is porous, necessitating an additional layer of immune control. **Shan *et al.*** (p. 447, published online 26 September; see the Perspective by **Belkaid and Grainger**) now report that in the small intestine, MUC2 plays an active role in immunological tolerance by activating a transcription factor in resident dendritic cells, thereby selectively blocking their ability to launch an inflammatory response. This work identifies MUC2 as a central mediator of immune tolerance to maintain homeostasis in the gut and possibly at other mucosal surfaces in the body.

Topological Replicas

When a periodic perturbation couples strongly to electrons in a solid, replicas of the original electronic levels are predicted to develop at certain energies—the so-called Floquet-Bloch states. Such conditions can be achieved by shining light on a solid, but the effect is challenging to observe. **Wang *et al.*** (p. 453) used time- and angle-resolved photoemission spectroscopy to photoexcite Bi₂Se₃ and observe its dispersion at various delay times. The replicas were seen at expected energy shifts, along with the gaps predicted to occur at the new energy-level crossings caused by the appearance of the replicas. Because Bi₂Se₃ is a topological insulator, the breaking of the time-reversal symmetry caused

by circularly polarized light resulted in the appearance of an energy gap at the Dirac point, indicating an interesting route toward manipulating electronic states in such materials.

Piecing Together Hydrogenase

Microbial hydrogenase enzymes generally use iron to catalyze the reversible formation of hydrogen from protons and electrons. Key to their efficiency is a set of iron-coordinating ligands, including CO and cyanide. **Kuchenreuther *et al.*** (p. 472) examined how the HydG maturase enzyme breaks down the amino acid tyrosine to derive these diatomic ligands for assembly of the diiron class of hydrogenases. The first step involves abstraction of an H atom from the phenolic OH substituent of the side chain. Electron paramagnetic resonance spectroscopy revealed a radical intermediate that subsequently results from heterolysis of the bond tethering the side chain to the α -carbon. With the side chain thus jettisoned, the residual dehydroglycine could be transformed into CO and CN⁻.

Exploiting Redundancy

The genetic code is redundant—multiple codons can code for the same amino acid. So-called synonymous codon changes within genes can nonetheless have substantial effects on protein expression, which have been attributed to changes in the structure of 5' messenger RNAs, among other factors. **Goodman *et al.*** (p. 475, published online 26 September) built and measured the expression of a synthetic library of 14,000 variant N-terminal sequences of 137

Escherichia coli genes to show that, unexpectedly, rare codons had a bigger effect on increasing protein expression than more common codons. Increased RNA structure downstream of translation initiation appeared to represent the major determinant of expression differences owing to codon usage.

Farming or Fishing

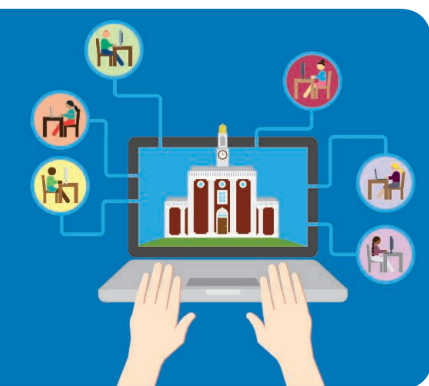
Evidence has been mounting that most modern European populations originated from the immigration of farmers who displaced the hunter-gatherers of the Mesolithic. **Bollongino *et al.*** (p. 479, published online 10 October) present



analyses of palaeogenetic and isotopic data from Neolithic human skeletons from the Blätterhöhle burial site in Germany. The analyses identify a Neolithic freshwater fish-eating hunter-gatherer group, living contemporaneously and in close proximity to a Neolithic farming group. While there is some evidence that hunter-gatherer women may have admixed into the farming population, it appears likely that marriage or cultural boundaries between the groups persisted for over two millennia. Thus, the transition from the Mesolithic involved a more complex pattern of coexistence among humans of different genetic origins and cultures in the Neolithic, rather than a more abrupt transition.

Bricks and MOOCs

PARENTS EVERYWHERE KNOW THAT A COLLEGE EDUCATION FOR THEIR CHILDREN IS KEY TO A prosperous future. At a recent Summit on Higher Education,* journalist Fareed Zakaria, the keynote speaker, recalled a conversation with the prime minister of a large developing nation who stated (only half in jest) that his strategy for accessible higher education was to provide broadband wireless network capability to every corner of his country and tell students to take advantage of free online offerings from U.S. universities—the massive open online courses (MOOCs). Mitch Daniels, president of Purdue University, responded to the topic of online learning with the question: “How can universities articulate the value proposition that will motivate students to move in for four years?” In his words, how can universities pass the “pajama test”? Will MOOCs exist as a parallel track alongside residential university experiences, creating new opportunities and new markets? Or are they a disruptive technology that will replace conventional universities, sending them the way of the record player or typewriter?



For research universities, much is at stake in this outcome. It is not possible to separate their higher-education mission from their research mission, and it is all undertaken within an experiential learning environment on campus. Online education does not substitute for hands-on training in the laboratory of an active researcher. Students become part of a research team to address problems that cannot be solved with the skills of one person alone. They see at first hand the applications of research ethics and scientific integrity, and they are inculcated with the culture of science—a process difficult if not impossible to replicate remotely. And yet even the research environment is changing. Many research teams are now collaborating over great distances, and members are challenged to cope with the interpersonal facets of team interactions when there is scant direct contact to build trust. A unique aspect of MOOCs is the interactive participation aimed at building a community for students and faculty. Experiencing team building through online courses could help tomorrow’s researchers learn the future skills they need in an increasingly distributed network of collaborators.

I believe that colleges and universities will survive the online learning revolution and perhaps even thrive as a result. The simple fact is that income increases with educational attainment, and by 2018, more than 60% of all jobs in the United States will require at least some college education.* Maintaining the affordability of college in the United States is therefore a core issue. Colleges and universities should embrace innovative online learning environments and determine how best to incorporate them with traditional classroom, laboratory, seminar, and field classes to reduce the cost of education, improve pedagogy, and increase the accessibility of higher education to all who seek it. Rather than resisting this movement, premier research universities in the United States have been at the forefront of the online learning revolution. They are creating much of the educational content and are discovering ways to best take advantage of online delivery methods to teach large numbers of students in interactive ways that are not possible in a conventional classroom format. For example, MOOCs can be effective vehicles for providing lifelong learning opportunities to keep the current workforce up to date with the latest career opportunities.

Online and traditional universities are connected for the foreseeable future. Without the bricks-and-mortar universities and their reputations behind these distance learning efforts, it is not clear that there is a viable business model to sustain online efforts. After all, the only continuously operating human institution older than the university system is the Vatican; universities have endured this long by remaining essential, and they will continue to be so.

— Marcia McNutt

*Sponsors of the summit were the Carnegie Corporation, the Bill & Melinda Gates Foundation, and the William and Flora Hewlett Foundation.



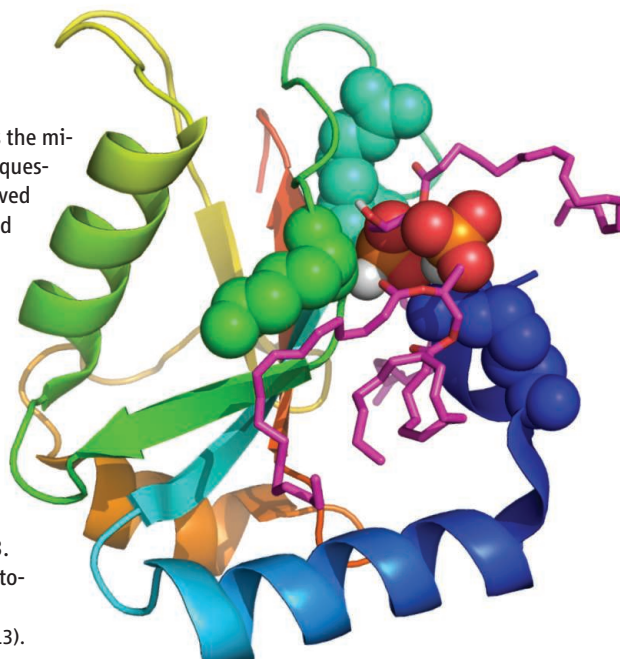
Marcia McNutt is Editor-in-Chief of *Science*.

CELL BIOLOGY

Eat Now

The daily wear and tear experienced by our cells can damage internal organelles, such as the mitochondria. The recognition and subsequent removal of injured mitochondria rely on a sequestration and engulfment process known as mitophagy, but the molecular pathways involved are unclear. Mitophagy plays a critical role in cellular health and in quality control, and its disruption has been implicated in neurodegeneration, diabetes, cardiac disease, and cancer. Working with cultured neuronal cells, Chu *et al.* now describe an “eat me” signal that identifies injured mitochondria as being fit for consumption by the intracellular recycling machinery. Cardiolipin is a mitochondria-specific phospholipid that normally resides within the inner mitochondrial membrane. The collapse of membrane asymmetry in mitochondria owing to damage inflicted by a variety of pro-mitophagy agents leads to the exposure of cardiolipin on the outer mitochondrial membrane, which marks them for delivery to lysosomes. The authors show that cardiolipin interacts with LC3, a protein known to be involved in autophagy; in particular, two arginine residues in the N-terminal region of LC3 stabilize the phosphate groups of cardiolipin, and subtracting the guanidinium side chains via mutation abrogated the binding of LC3. Thus, cells protect themselves from the potentially deleterious effects of damaged mitochondria by exploiting the damage-induced redistribution of cardiolipin. — SMH

Nat. Cell Biol. **15**, 1197 (2013).



NEUROSCIENCE

Sleep Now

Neuropeptide Y (NPY) acts in the mammalian brain to control numerous behaviors, including food intake and sleep. In *Drosophila*, there are two NPY-like peptides, neuropeptide F (NPF) and also a short version (sNPF). Shang *et al.* have found that the activation of sNPF-expressing neurons in the fly brain promotes sleep. When the activity of these neurons was enhanced, flies were prone to sleep more; later, when these neurons were silenced, flies actually slept less, as if their internal clocks had registered the additional time already spent sleeping. Conversely, experimentally activating these neurons during a period of sleep deprivation (achieved by incessant agitation on a vortex mixer) reduced the amount of catch-up sleep needed. These neurons are controlled by wake-promoting neurons that express the neurotransmitter γ -aminobutyric acid and suppress the activity of the sNPF neurons, reducing daytime sleep. Finally, the effects of sNPF on feeding appear to be secondary to its effects on sleep. — LDC

Neuron **80**, 171 (2013).

CLIMATE SCIENCE

Historical Carbon Uptake

The high levels of atmospheric CO₂ that have resulted from fossil fuel burning and other anthropogenic activities over the past 150 years are expected to cause increased uptake of carbon by the terrestrial biosphere over the next century, thereby partially offsetting some

of the CO₂ emissions. This effect is difficult to quantify, though. Deforestation and other land-use changes have transferred great quantities of carbon from the biosphere to the atmosphere since preindustrial times, making the magnitude of carbon uptake by land plants difficult to infer. Shevliakova *et al.* address this issue with a coupled climate–carbon cycle model study



of the terrestrial carbon sink, paying special attention to how land use has changed since the beginning of the industrial revolution. They estimate that enhanced vegetation growth over that period reduced atmospheric CO₂ concentration by 85 ppm below what it would have been without that effect, thereby avoiding approximately 0.3°C of warming. This represents a dramatic shift of the carbon budget, by more than 250 billion tons of carbon—more than 30 years of emissions at current rates. — HJS

Proc. Natl. Acad. Sci. U.S.A. **110**, 16730 (2013).

MATERIALS SCIENCE

Tracking Graphene Growth

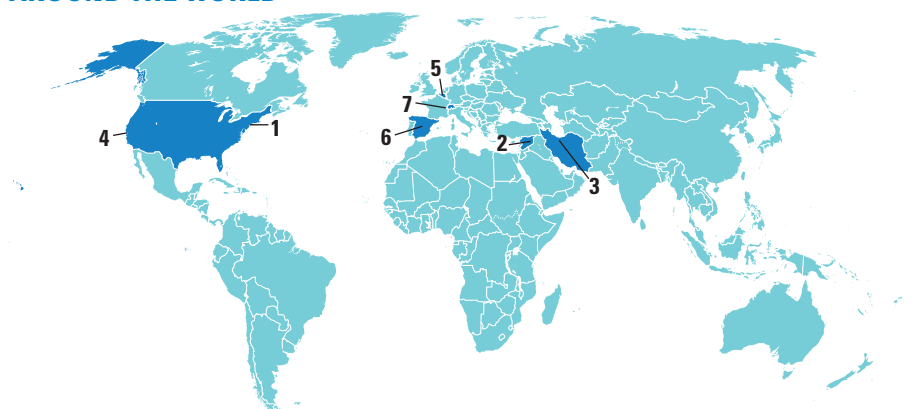
A convenient route for preparing monolayer graphene is via chemical vapor deposition of hydrocarbons onto copper substrates held at elevated temperatures. Kidambi *et al.* examined how changes in process conditions affect the

growth chemistry with several in situ techniques, including x-ray photoelectron spectroscopy (XPS) and environmental scanning electron microscopy (ESEM). They used benzene as the hydrocarbon source (at partial pressures up to ~4 torr) at substrate temperatures of 900°C. During growth in the absence of air, a shift in the main C1s XPS peak indicated that graphene was coupled to metallic copper. After cooling in vacuum and air exposure, the main carbon feature underwent

a shift indicative of decoupling of the graphene from the surface via oxygen intercalation, rather than surface oxidation. Visualization of this decoupling with ESEM revealed an island-growth process proceeding from the edges of the island inward. However, if air was present during growth, there was an increase in the carbon peak associated with carbon deposition at defect sites; surface oxide formed, and roughly half of the graphene present was decoupled from the surface. — PDS

Nano Lett. **13**, 4769 (2013).

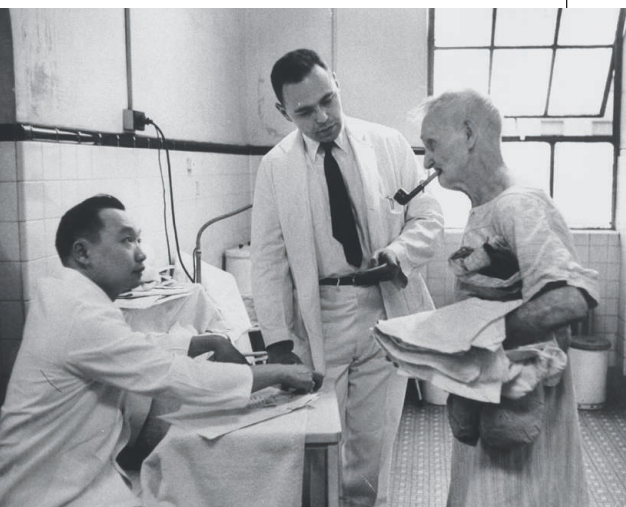
AROUND THE WORLD



New York City 1

Papers Condemn '50s Cancer Research

A medical historian chronicled a shameful chapter in U.S. medical history last week, detailing in two publications how 1200 homeless alcoholics in a Manhattan neighborhood were offered a few days of food in exchange for a biopsy of their prostate. The papers by Robert Aronowitz of the University of Pennsylvania, published in the *American*



Health exchange. A Bowery resident sees doctors at New York's Francis Delafield Hospital in 1957.

Journal of Public Health and the *Bulletin of the History of Medicine*, describe the "Bowery series," studies named after the neighborhood where the men lived that began in 1951 and ran more than a decade. Researchers from Columbia University recruited the men "and subjected them to many invasive tests and procedures," Aronowitz writes. Many of the men also suffered complications, including rectal lacerations

and incontinence. He notes that despite its ethical flaws, the series presaged the "early detection and radical treatment paradigm" adopted for prostate cancer decades later.

Deir Al Zour, Syria 2

Suspected Polio Outbreak in Syria

After a 14-year hiatus, polio is back in Syria, health officials fear. As of 21 October, 22 cases of acute flaccid paralysis had been reported in Deir Al Zour, one of the contested areas near the Iraq border. Although lab confirmation was still pending, the World Health Organization (WHO) and the government were treating the cases as "hot" because they meet the clinical and epidemiologic criteria for polio, including asymmetrical paralysis and clustering within a geographical area. "This one ticks all the boxes," says Bruce Aylward, who leads the Global Polio Eradication Initiative from WHO. An emergency vaccination campaign is planned for as early as 27 October, to be followed by a coordinated campaign across seven countries in November or December. Wild polio was last seen in Syria in 1999. The country "had a fantastic vaccination program, very strong public health," Aylward says. This apparent relapse reflects "the deterioration in health services in the face of the crisis."

Tehran 3

Rouhani Promises Academic Freedom

After a long, dark night, a new dawn may have arrived for Iranian academia. In a speech last week at the University of Tehran, Iranian President Hassan Rouhani



New era? Iranian President Hassan Rouhani at the University of Tehran last week.

called for greater academic freedom on university campuses and looser regulations on overseas travel.

His predecessor, former Iranian President Mahmoud Ahmadinejad, saw academia as a hotbed for discontent and sought to expunge liberal elements during his administration's 8 years in power. Last autumn, for instance, Iranian news agencies reported that former science minister Kamran Daneshjoo had ordered universities to purge open-minded faculty to "prevent secular lecturers from trying to disseminate anti-religion beliefs."

What a difference a year makes. "The security atmosphere [on campuses] must be relaxed," caretaker science minister Jaafar Towfiqi told *Asharq Al-Awsat* newspaper last month. Rouhani expanded on that theme last week. "I will ask the intelligence ministry to trust academia," *Asharq Al-Awsat* quoted him as saying. "The security environment will lead to hypocrisy and we do not want that."

Palo Alto, California 4

Replication Project Scores \$1.3 Million

The Reproducibility Initiative, which aims to repeat the results of published research, has received \$1.3 million from the Laura and John Arnold Foundation, which it said last week will fund an effort to reproduce 50 landmark cancer studies published between 2010 and 2012. The initiative was launched last year by breast cancer biologist Elizabeth Iorns to address a widely acknowledged problem in biomedical research: Many published studies can't be repeated, and many researchers aren't enthusiastic about replicating existing work. So far, the initiative has relied in part on authors to pay to have their work reproduced, but with the

CREDITS (TOP TO BOTTOM): EBRAHIM SEYEDI/PRESIDENCY OFFICE/AP PHOTO; PHOTO BY WALTER SANDERS/TIME LIFE PICTURES/GETTY IMAGES

Downloaded from www.sciencemag.org on October 24, 2013

THEY SAID IT

“Non-sarcastic hugs to those at NASA who will be returning to their jobs as awesome science wizards of the stars.”

—Tweet on 16 October from @SarcasticRover, the Mars Curiosity rover’s unofficial alter ego, after news that the government shutdown had ended.

new money infusion, it can target the most influential studies for scrutiny. The online marketplace Science Exchange, which Iorns co-founded in 2011, will farm out the experiments to various companies, and *PLOS ONE* has pledged to make the results freely available. <http://scim.ag/Reprod>

Brussels 5

Manifesto Calls for Borderless European Science

Two members of the European Parliament have proposed legally binding measures to create the European Research Area (ERA), making it easier for scientists to work with colleagues across borders and relocate within the European Union. In a manifesto presented last week, former Italian science minister Luigi Berlinguer and Amalia Sartori, chairwoman of the European Parliament’s research committee, said the time has come to “speed up” the advent of the ERA through E.U. directives or even a “constitutional commitment.” The ERA idea has been discussed for more than a decade, but progress has been slow. Berlinguer and Sartori argue legal measures are needed to coordinate national research programs, improve cross-border arrangements for pension and social security rights, and make research grants portable from one country to another. Six other Parliament members have endorsed the manifesto, but many universities and research funders favor a nonbinding, voluntary approach. Concrete action is unlikely until after the European Parliament elections in May. <http://scim.ag/ERAmami>

Science LIVE

Join us on Thursday, 31 October, at 3 p.m. EDT for a live chat exploring the science of fear. <http://scim.ag/science-live>



Big Break

Scientists have recovered the largest fragment to date of the 10,000-metric-ton meteor that exploded over the Russian city of Chelyabinsk in February. While the meteorite hunters were hauling their find out of Lake Chebarkul, however, the alien boulder broke into three pieces. Photographers and onlookers encircled the chunks of damaged space rock as they were clipped onto a steelyard balance, which displayed “570 kg” ... and then broke as well. The fragment is an exciting find, says Mark Boslough, a physicist at Sandia National Laboratories in Albuquerque, New Mexico, who predicts that “this will be the lion’s share of the total mass recovered.” Although it might not change estimates of the parent body’s orbit and trajectory, “it will give us more confidence that what we have is correct,” he says.

Madrid 6

€70 Million Will Keep Spanish Science Afloat

Spain’s government last week agreed to a €70 million (\$96 million) cash injection to save the Spanish National Research Council (CSIC) from imminent bankruptcy. The fresh money ends months of uncertainty about the immediate future of Spain’s largest research organization, which has faced financial woes since 2009, largely due to across-the-board funding cuts. The infusion allows CSIC “to face the immediate future with a greater optimism,” CSIC President Emilio Lora-Tamayo wrote in a letter to CSIC staff.

A 2014 draft budget bill calls for increased spending on civilian research, and the government also plans to increase CSIC’s annual lump-sum operating funding by about €50 million (\$69 million). But Carlos Andradas Heranz, a mathematician at the Complutense University of Madrid and president of the Confederation of Spanish Scientific Societies, predicts that Spanish research will continue to face funding pressures.

The cash injection came one day after scientists at many public research organizations

and universities held “a mourning day for science,” asking the government to further boost research spending. <http://scim.ag/CSICcash>

Geneva, Switzerland 7

WHO Sounds Alarm On Drug-Resistant TB

The World Health Organization (WHO) has added an alarm bell to its assessment of multidrug-resistant tuberculosis (MDR TB), calling it “a public health crisis” in an annual update on the disease, released on 23 October.

WHO has declared for nearly 2 decades that tuberculosis, which sickened 8.6 million in 2012, is “a global public health emergency.” Mortality rates have declined in drug-susceptible cases, which are curable with a 6-month course of three well-tolerated drugs. But in the new assessment, WHO scolds countries for being “far off track” in the diagnosis and treatment of MDR TB, which requires giving patients more toxic drugs for up to 2 years. WHO estimates that 450,000 people developed MDR TB last year, but only 94,000 of the cases were detected, and 82% of these received treatment.

NEWSMAKERS

CIRM Director Steps Down

Alan Trounson, the Australian scientist who has headed California's \$3 billion stem cell institute for nearly 6 years, is stepping down to be closer to his family.



Trounson

Trounson, 67, a leading in vitro fertilization researcher, left Monash University in Melbourne, Australia, to become president of the California Institute for Regenerative Medicine (CIRM) in

late 2007. He oversaw the agency's shift from basic research and new buildings to projects aimed at moving discoveries to the clinic. Overall, CIRM has disbursed \$1.9 billion in grants. During his tenure, CIRM also went through various controversies, including the abrupt departure of two of its leaders.

"I have loved working at CIRM ... but ultimately it came down to a choice between CIRM and a life including

my family," Trounson stated in a press release. He has four children in Australia.

Trounson leaves at a challenging time for the agency, which will run out of its funding from state bonds in 2017. He has agreed to stay on while the CIRM board searches for his successor.

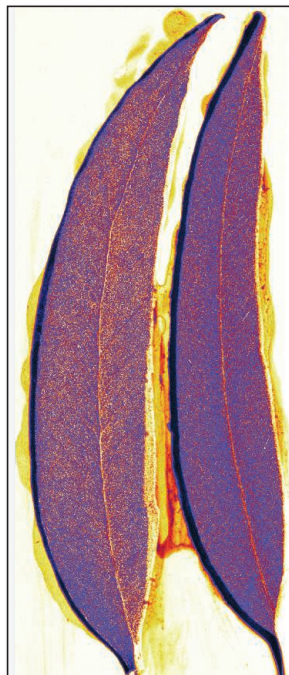
http://scim.ag/_CIRM

FINDINGS

Money Does Grow On Trees

Prospectors, take note: Trees growing over deeply buried deposits of gold ore sport leaves with higher-than-normal concentrations of the glittering element.

Eucalyptus trees above a 35-meter-deep deposit in southern Australia had 20 times more gold in the gummy substances coating their leaves than did trees that grew 800 meters away, Mel Lintern, a geochemist at the CSIRO Earth Science and Resource Engineering division in Kensington, Australia,



Gold bush. Eucalyptus trees over a gold deposit had elevated calcium (*shown*) and gold, both drawn from the soil.

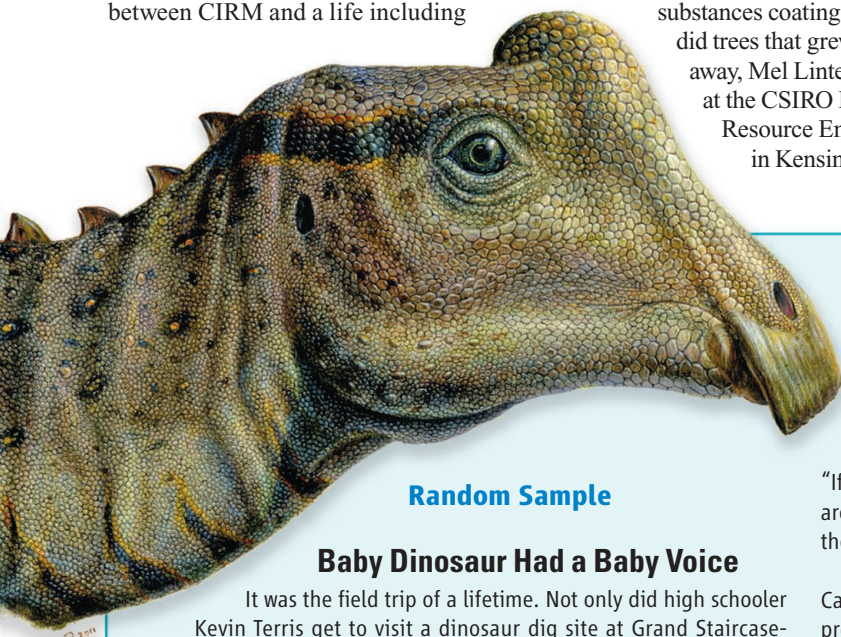
and colleagues report in this week's issue of *Nature Communications*.

While previous studies have noted anomalously high gold concentrations, they couldn't rule out the possibility that tiny particles of the element had become stuck to leaves after being carried by wind. So Lintern's group also grew seedlings in laboratory greenhouses insulated from airborne dust, watering them with gold-tainted solutions to show that trees actually transport the element from

soil into their leaves.

The analysis provides "a conclusive set of evidence ... from a very nicely constructed set of experiments," says Clifford Stanley, a geochemist at Acadia University in Wolfville, Canada. "The tree is a conveyor belt bringing gold to the surface."

<http://scim.ag/GoldTree>



Random Sample

Baby Dinosaur Had a Baby Voice

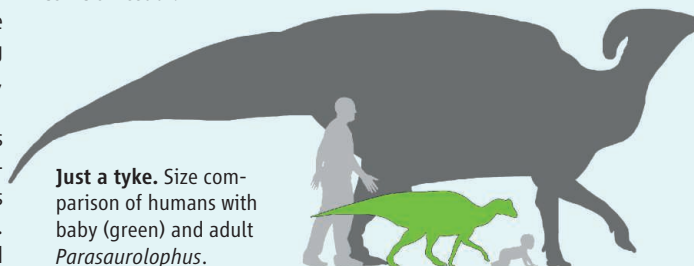
It was the field trip of a lifetime. Not only did high schooler Kevin Terris get to visit a dinosaur dig site at Grand Staircase-Escalante National Monument, but the bone he spotted—after experienced paleontologists walked right past it—revealed the most complete skeleton ever found of *Parasaurolophus*, a duck-billed, plant-eating dinosaur from the Late Cretaceous. It's also the smallest: The dino, nicknamed "Joe," is a baby.

Parasaurolophus adults bore impressive headgear. Scientists believe they blew into their enormous crests like trumpets to vocalize. "One of the standing questions has been, how did it grow this big crest?" says Andrew Farke, a paleontologist at the Raymond M. Alf Museum of Paleontology in Claremont, California, who is the lead

author on a study of the new skeleton, published in the journal *PeerJ* this week.

Some dinosaur species didn't start growing their ornamentation until puberty, but baby Joe, less than a year old when he died, already had a small, hollow bump on his head. Farke's team calculates that he would have called with a high-pitched baby voice, which may have helped other dinosaurs identify him. "If you're a baby and you hear a big, booming call, you know the adults are nearby. If you're an adult and you hear little squeakers, you know the young are nearby."

Paleontologist David Evans of the Royal Ontario Museum in Toronto, Canada, who was not involved with the research, agrees with that interpretation and says Joe is "a truly remarkable specimen of a rare and iconic dinosaur."



Just a tyke. Size comparison of humans with baby (green) and adult *Parasaurolophus*.

GENOMES

Ancient DNA Links Native Americans With Europe

SANTA FE—Where did the first Americans come from? Most researchers agree that Paleoamericans moved across the Bering Land Bridge from Asia sometime before 15,000 years ago, suggesting roots in East Asia. But just where the source populations arose has long been a mystery.

Now comes a surprising twist, from the complete nuclear genome of a Siberian boy who died 24,000 years ago—the oldest complete genome of a modern human sequenced to date. His DNA shows close ties to those of today's Native Americans. Yet he apparently descended not from East Asians, but from people who had lived in Europe or western Asia. The finding suggests that about a third of the ancestry of today's Native Americans

can be traced to “western Eurasia,” with the other two-thirds coming from eastern Asia, according to a talk at a meeting* here by ancient DNA expert Eske Willerslev of the University of Copenhagen. It also implies that traces of European ancestry previously detected in modern Native Americans do not come solely from mixing with European colonists, as most scientists had assumed, but have much deeper roots.

“I’m still processing that Native Americans are one-third European,” says geneticist Connie Mulligan of the University of Florida in Gainesville. “It’s jaw-dropping.” At the very least, says geneticist Dennis O’Rourke of the University of Utah in Salt Lake City, “this is going to stimulate a lot of discussion.”

Researchers have been trying to parse the origins of the first Americans for decades. Most agree that people moved across Beringia, via a vast ice age land bridge (see map p. 410), and began spreading through the Americas, reaching Chile by 14,500 years ago. But the origins of the source popula-

tions are not clear, and some archaeologists have even suggested that ancient Europeans crossing the Atlantic were part of the mix (*Science*, 16 March 2012, p. 1289). Others have contended that early skeletons found



Boy's bones. DNA from this ancient Siberian skeleton offers clues to the first Americans.

in the Americas, such as the 9000-year-old Kennewick Man, show some European features (*Science*, 10 April 1998, p. 190). In his talk, Willerslev argued that the ancient genome “can actually explain a lot of these inconsistencies,” by offering glimpses of prehistoric populations before more recent migrations and other demographic events blurred the picture.

The genome comes from the right upper arm bone of a boy aged about 4 years, who lived by Siberia's Belaya River. Those who buried him adorned his grave with flint tools, pendants, a bead necklace, and a sprinkling of ochre. In the 1920s, Russian archaeologists discovered the burial and other artifacts near a village called Mal'ta, which gave the celebrated site its name. Willerslev and co-author Kelly Graf of Texas A&M University in College Station, traveled to the State Hermitage Museum in St. Petersburg, Russia, where the boy's remains are housed, and took a bone sample.

Willerslev reported that the team was able to sequence the boy's genome, and also

to radiocarbon date the bone. The team then used a variety of statistical methods to compare the genome with that of living populations. They found that a portion of the boy's genome is shared only by today's Native Americans and no other groups, showing a close relationship. Yet the child's Y chromosome belongs to a genetic group called Y haplogroup R, and its mitochondrial DNA to a haplogroup U. Today, those haplogroups are found almost exclusively in people living in Europe and regions of Asia west of the Altai Mountains, which are near the borders of Russia, China, and Mongolia.

One expected relationship was missing from the picture: The boy's genome showed no connection to modern East Asians. DNA studies of living people strongly suggest that East Asians—perhaps Siberians, Chinese, or Japanese—make up the major part of Native American ancestors. So how could the boy be related to living Native Americans, but not to East Asians? “This was kind of puzzling at first,” Willerslev told the meeting.

But there seemed little doubt that the finding was correct, he said, because nearly all Native Americans from North and South America were equally related to the Mal'ta child, indicating that he represented very deep Native American roots.

The team proposes a relatively simple scenario: Before 24,000 years ago, the ancestors of Native Americans and the ancestors of today's East Asians split into distinct groups. The Mal'ta child represents a population of Native American ancestors

who moved into Siberia, probably from Europe or west Asia. Then, sometime after the Mal'ta boy died, this population mixed with East Asians. The new, admixed population eventually made its way to the Americas. Exactly when and where the

admixture happened is not clear, Willerslev said. But the deep roots in Europe or west Asia could help explain features of some Paleoamerican skeletons and of Native American DNA today. “The west Eurasian [genetic] signatures that we very often find in today's Native Americans don't all come from postcolonial admixture,” Willerslev

Online

sciencemag.org

Podcast interview with author Michael Balter (http://scim.ag/pod_6157).

* Paleoamerican Odyssey, Santa Fe, 16–20 October.

said in his talk. “Some of them are ancient.”

The talk sparked lively exchange, and not everyone was ready to buy the team’s scenario, at least until they can read the full paper, which is in press at *Nature*. “This is a lot to hang on one skeleton,” Mulligan says. Willerslev said during the discussion that his group is now trying to sequence the genomes of skeletons “further west.”

The new findings are consistent with a report published in *Genetics* last year (and almost entirely ignored at the time) that used modern DNA to conclude that Native Americans have significant—and ancient—ties to Europeans. “Our group is very excited to see this,” says Alexander Kim, who works with geneticist David Reich at Harvard Medical School in Boston and represented the group at the meeting. Reich’s team found that populations they identified as Native American ancestors in Asia apparently also contributed genes to populations in northern Europe.



Headed east. The Mal'ta boy was related to people who later migrated across Beringia to the Americas.

Thus, both studies suggest a source population in Asia whose genes made their way east all the way to the Americas, and west, all the way to Europe.

“Mal'ta might be a missing link, a representative of the Asian population that admixed

both into Europeans and Native Americans,” Reich says. If so, he adds, it shows “the value of ancient DNA in peeling back history and resolving mysteries that are difficult to solve using only present day samples.”

—MICHAEL BALTER

SCIENCE FUNDING

U.S. Shutdown Ends, but Not Budget Anxiety

And now for the main event.

The reopening of the U.S. government last week after a 16-day shutdown has allowed most federal researchers to get back to work and has restarted the grantmaking machinery at key science funding agencies. But the anxiety isn't over.

Business as usual means Congress must again confront the unpleasant political reality that the federal government spends hundreds of billions of dollars more each year than it takes in. Legislators have given themselves 2 months to come up with at least the outline of a plan to begin reducing the deficit, starting with a budget for the 2014 fiscal year that began on 1 October. And as that effort unfolds, scientists need to know that the rules of the federal budget game have changed.

The good news for research advocates: The rigid, across-the-board budget cuts that shrunk most agency budgets by 5% in the 2013 fiscal year are gone. The mechanism that spawned them, sequestration, remains in effect. However, instead of applying a meat ax to every federal agency, sequestration has morphed into a ceiling on all federal discretionary spending—outlays for every-

thing other than mandatory programs such as Medicare and Social Security, and interest on the national debt. And that ceiling is likely to remain flat in 2014 for the pot of federal funding that includes fundamental research.

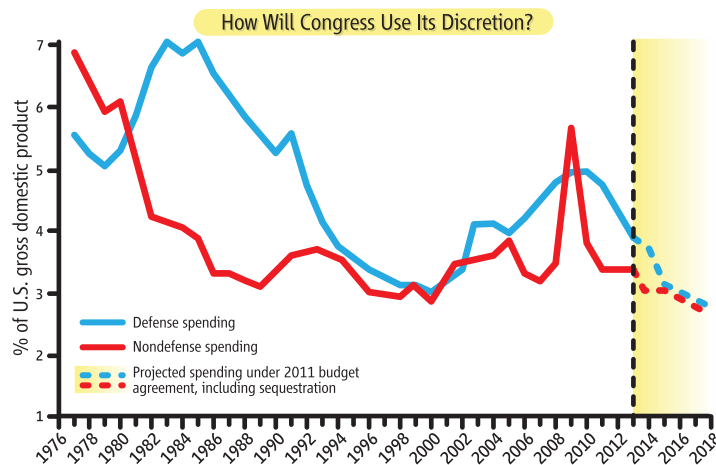
that its ultimate payoff can't be predicted. The bad news, however, is that science is back to competing against every other special interest for a bigger slice of a federal funding pie that isn't growing. “It will be up to the research community to make the case that science is essential within the constraints that legislators are facing,” says Samuel Rankin, head of the Coalition for National Science Funding and head of the Washington, D.C., office of the American Mathematical Society.

The temporary budget approved last week, which funds the government until 15 January at 2013 levels, sets overall discretionary spending at \$986 billion. Defense spending accounts for \$518 billion, leaving \$468 billion for civilian programs.

Absent a new agreement, in January the discretionary total for 2014 will shrink by nearly \$20 billion, to \$967 billion.

Defense spending, which was protected from deeper cuts in 2013, would absorb the entire blow, dropping by 4% to \$498 billion. Spending for civilian programs would stay essentially level, at \$469 billion.

The lower 2014 spending limits are part of a 2011 budget agreement to reduce the



Fear of falling. The budget agreement squeezes civilian discretionary funding, which spiked because of the 2009 stimulus package after holding steady for decades as a share of GDP.

That change means Congress and the White House will have more flexibility to decide where to make any cuts. So researchers will have a chance to make the case that research is a long-term investment, that it generates the innovations needed for sustained economic growth and prosperity, and

CREDITS (TOP TO BOTTOM): G. GRULLÓN/SCIENCE; ADAPTED FROM CONGRESSIONAL RESEARCH SERVICE

ASTRONOMY

Earliest Known Galaxy Formed Stars at a Breakneck Pace

deficit over the next decade. Specifically, Congress and the White House agreed to automatically shrink federal spending if policymakers couldn't find a better way to get the job done. Under the law, the sequestration cuts are supposed to apply equally to defense and civilian spending. Both spending categories start to creep up in 2015, however, and continue rising through 2021.

Both political parties want to end sequestration but are at odds about how to proceed. Many Republicans, for instance, have called for softening the upcoming defense cuts by trimming social programs, but are opposed to imposing new taxes to raise revenue. In contrast, President Barack Obama and congressional Democrats are pushing for a "balanced approach," that is, some combination of spending cuts and tax increases. Most science advocates favor that approach, which they believe will eventually lead to somewhat higher spending on science.

"We urge Congress to use the next three months to develop a workable, long-term budget framework that deals with the nation's debt in a way that allows smart investments in such things as scientific research and education," a coalition of leading public and private research universities declared after the government returned to work. "As long as sequestration remains in effect, our economic recovery remains in jeopardy."

Hunter Rawlings, president of the 62-member Association of American Universities, which is part of the coalition, says one major impediment to increased science spending is the continued growth of so-called entitlement programs, such as Social Security and Medicare. Another is the budget process itself. "Members of Congress do not vote against research," he notes. "But they let cuts in research happen because of a budget process that takes money away from innovation and puts it in the hands of old people like me, who frankly should not be getting the percentage of revenue that is coming to our generation."

But few legislators are willing to tinker with politically sensitive entitlements. And many observers are betting the partisan gridlock over new taxes and spending cuts will continue, with lawmakers opting for a continuing resolution (CR) that simply extends funding at current levels—and avoids the big questions for another year.

"Congress can meet the spending caps as it chooses," says Richard Kogan of the Center on Budget and Policy Priorities, a non-partisan think tank in Washington, D.C. "But as long as there's gridlock, [a CR] is probably where you'll end up." —JEFFREY MERVIS

In the beginning was the big bang; then, hundreds of millions of years later, the universe was full of galaxies. This week, astronomers report taking another step into the unexplored time in between: They have imaged the earliest galaxy yet, dating from just 700 million years after the big bang. It is aglow with hot, newborn stars, the researchers say, pointing to a rate of star formation that they estimate to be a hundred times that of the modern Milky Way. Reported in *Nature* this week, the find may offer a glimpse of an unexpected period of frenetic star birth in the early universe.

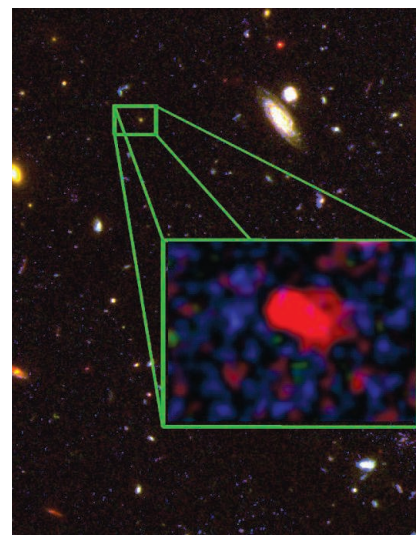
The galaxy was one of dozens imaged in a Hubble Space Telescope survey designed to pick up faint, distant galaxies. Their reddish color suggested they were remote enough for the expansion of the universe to stretch and redden their light. But astronomers need to study a galaxy's spectrum to determine its precise distance from Earth.

Researchers led by Steven Finkelstein, an astronomer at the University of Texas (UT), Austin, looked at a sample of these galaxies through a new spectrograph installed on one of the two 10-meter Keck telescopes at Mauna Kea, Hawaii. The instrument—called the Multi-Object Spectrometer for Infra-Red Exploration—can analyze light from 45 objects simultaneously. Traditional spectrographs look at only one object at a time.

Studying 43 galaxies over two nights of observations, Finkelstein and his colleagues detected a near-infrared emission line from one of the galaxies, from which they measured a redshift of 7.51—a measure of how much the light waves had been stretched by cosmic expansion. That meant the galaxy was 13.1 billion light-years away, in a universe 13.8 billion years old.

Because the galaxy was much brighter than distant galaxies typically are, Finkelstein and his colleagues could make inferences about it from its image and its spectrum. For one, the galaxy seems to be significantly rich in "metals"—elements heavier than hydrogen and helium. Because all of those elements originate from fusion reactions in the heart of stars and are spewed out when those stars explode as supernovae, the relatively high metallicity of the galaxy suggests that it had already seen the birth and death of generations of stars by the time the universe was 700 million years old.

The researchers also inferred from its brightness that the galaxy was forming stars at a rate of 330 solar masses per year. That is surprising, Finkelstein says, because other galaxies nearly as distant typically form stars at a rate no higher than 20 to 30 solar masses per year. Volker Bromm, a theoretical astrophysicist at UT Austin who wasn't part of Finkelstein's team, says the finding could mark a period in the cosmic timeline when galaxies went from form-



Record setter. Light from this galaxy shows it existed a mere 700 million years after the big bang—the earliest such cosmic object detected so far.

ing relatively few stars to bursting with star formation. "There may be an early 'cosmic bottleneck' that limits the efficiency of star formation," he says. Perhaps, he says, when a galaxy is sufficiently enriched with "metals"—which help gas clouds coalesce more easily into stars—the star formation rate accelerates considerably.

Not everybody, however, is convinced that the ancient galaxy was as active a star factory as Finkelstein and his colleagues infer. Garth Illingworth, an astronomer at the University of California, Santa Cruz, says the models the authors relied on to estimate star formation rates might not work well for such far-off galaxies. "The star formation rate would still be higher than fainter galaxies at that redshift, and it can be said that a lot of star formation is taking place, but I doubt that it is nearly as extreme as they say," he says.

—YUDHIJIT BHATTACHARJEE

U.K. Science Adviser Faces Emotionally Charged Issues and Uncertain Science

LONDON—Since becoming the U.K. government's chief science adviser in April, Mark Walport, 60, has had to untangle some knotty scientific problems for Prime Minister David Cameron and his Cabinet. But interpreting the latest report from the U.N. Intergovernmental Panel on Climate Change (IPCC) and weighing conflicting evidence on whether badgers spread tuberculosis to cattle pale compared to the backlash he aroused just after taking up his post, when he declared in the *Financial Times* that he opposed a European ban on insecticides suspected of harming bees (*Science*, 10 May, p. 674). Last week, the medical scientist and former director of the Wellcome Trust, the United Kingdom's main biomedical research foundation, spoke with *Science* Editor-in-Chief Marcia McNutt and U.K. Deputy News Editor Daniel Clery about the challenges of bridging the gap between politicians and scientists. The transcript has been edited for clarity and brevity.

Q: Your public statements about neonicotinoid pesticides and their impact on bees landed you in hot water.

M.W.: There were some vocal critics. The issue is the distinction between hazard and risk. Of course neonicotinoid pesticides are hazardous. The question is, at the exposure levels in the agricultural environment do they harm insects they shouldn't. Frankly, the evidence is not as good as one would like, but the position has not changed to the point that it's appropriate to stop using them. The answer is that more work needs to be done. There were a small number of very vocal conservationists who felt that wasn't the right answer, but I was giving a scientific opinion.

Q: But isn't your view that no action needs to be taken on the use of neonicotinoids a policy statement rather than a scientific one?

M.W.: In the context of a European ban, it did not seem an appropriate scientific decision to stop the use of neonicotinoids without further work being done. You can debate whether that crosses the boundary or not, but my judgment was that it didn't.

Q: The papers showing how to make H5N1 bird flu more transmissible were a hot-button issue in the United States 2 years ago. What would your advice have been had these papers come from the United Kingdom?

M.W.: Let's do an imaginary experiment in which someone finds a way to mix two chemicals together to make a deadly gas. Would you publish the recipe? I think most



Out of the frying pan ... After a decade funding research as director of the Wellcome Trust, medical scientist Mark Walport must now interpret it for politicians.

people would say you wouldn't. So having established that as a principle, you have to look on a case-by-case basis.

You have to do that by a concordat, because everyone is responsible in the system. It starts with the scientists: They have to think about the consequences of the work they do. It comes to the people that employ the scientists; it comes to the funders; and it's the responsibility of the journal editors as well. So there are a whole series of fail-safes, but it is very difficult to write a set of rules and equally it would probably be disproportionate to set up an office of scrutiny that every paper would have to go through. It's about the use of judgment and proportionality.

Q: The U.K. government is currently conducting a trial cull of badgers despite scientific advice suggesting that culls are not effective at reducing the spread of tuberculosis in cattle. Is this an area where science advisers ought to be campaigning more vociferously?

M.W.: The issue is to make sure that the best scientific advice on the best evidence available is provided for ministers at all times, and to make sure they've seen it. One of the problems is that a lot of the very difficult decisions made by politicians are made at a time when there is uncertainty, and that's one of the very difficult things about being a scientific adviser. In the case of bovine tuberculosis, we

know that it's on the increase; there is evidence that badgers transmit it; [an earlier] study showed a 16% reduction [in bovine tuberculosis incidence] if you cull in an area of 150 square kilometers over a number of years. That is the evidence upon which ministers made a difficult decision [to proceed with the trial cull].

I'm struck that the depth of the evidence base for plant and animal diseases is rather weaker than the evidence base for human health. The challenge for me is to try and increase the quality of the evidence base so that when ministers are faced with making decisions in the face of uncertainty, that we do the best we can to minimize [uncertainty].

Q: The recent IPCC report is unambiguous in its warnings about the dangers of climate change, and yet we're still seeing rising public skepticism and a mismatch between policy and what needs to be done. What can scientists do?

M.W.: This is something I'm spending a huge amount of my time on at the moment, probably more than anything else. There are three challenges: climate science, although that's hard, it's probably the easiest bit; communication, and I don't think the scientific community has necessarily helped itself; and the policies that follow, which is the hardest of all.

Not being a specialist climatologist means I can ask climatologists questions and challenge the jargon. What the hell is negative emissions technology? We need to call it greenhouse gas removal. There is something in the jargon that the scientific community takes for granted that simply doesn't communicate. We have to get better and better and better at doing it.

INFECTIOUS DISEASE

Immune Suppressant Unexpectedly Boosts Flu Vaccine

Vaccine researchers are in the business of revving up the immune system to fight disease. So they are understandably perplexed by a result published online this week in *Nature Immunology*: Scientists gave mice a drug normally used to *suppress* the immune system—and found that it *bolstered* the powers of an influenza vaccine.

Led by immunologist Maureen McGargill of St. Jude Children's Research Hospital in Memphis, Tennessee, the team gave rapamycin, which helps prevent immunologic rejection in kidney transplant patients, to mice before immunizing them. The result: The mice produced a broader array of antibodies, defeating strains of the influenza virus that differed dramatically from the one used in the vaccine. The finding suggests a novel path to a long-sought “universal” flu immunization that can protect against many variants. It may also offer a way to elicit more effective antibody responses against myriad other diseases.

Results in mice, of course, often don't translate into humans, and rapamycin, a potent drug that has a somewhat murky mechanism of action, can have serious side effects. Still, the finding has immunologists talking. “It's really cool—and pretty counter-intuitive,” says Patrick Wilson, an immunologist at the University of Chicago in Illinois. “This is going to be my next journal club paper.”

McGargill and co-workers envisioned a different role for rapamycin when they set out to explore how the strain-specific influenza vaccines used today—which must be updated each year to keep up with the ever-changing virus—might be transformed into a universal shot. They sought ways to bolster one of the immune system's two virus-fighting arms: “cytotoxic” T cells—designated as CD8s because of receptors on their surfaces—which destroy cells that a virus has managed to infect. Antibodies, the immune system's other arm, target viruses directly and may work against only one strain of influenza, but CD8 T cells are less discriminating and can target many viral variants. In 2009, a team of scientists had found that rapamycin ramps up the response of CD8 T cells in both mice and monkeys, and McGargill and co-workers thought

that property could be key to a universal flu vaccine.

In the new study, the researchers gave mice rapamycin and an influenza vaccine against a strain known as H3N2 that com-

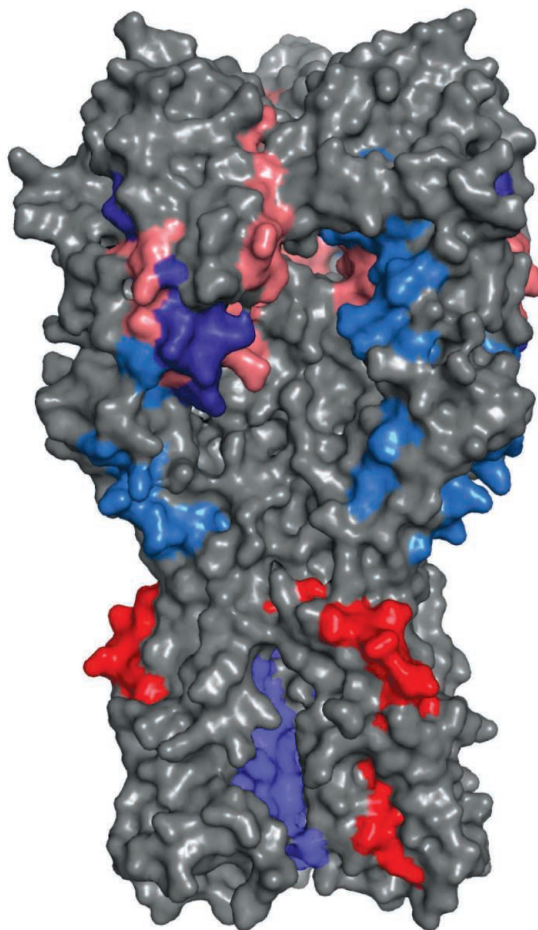
nologist at her institution who won the Nobel Prize in physiology or medicine for helping to elucidate how CD8s kill virally infected cells.

Instead, the researchers were astonished to find that an altered antibody response explained the protection. Through a series of complicated experiments, they found that the drug inhibited a molecule known as the “mammalian target of rapamycin.” That, in turn, effectively aborted the normal antibody maturation process, a kind of conversation between the immune system and the virus that leads to highly specific antibodies. The result was to free B cells to make higher-than-normal levels of antibodies that were less specific but had a broader reach, capable of neutralizing many viral variants.

The finding is just the latest surprise from rapamycin, says immunologist Rafi Ahmed of Emory University in Atlanta, author of the 2009 *Nature* study that inspired the work. Discovered from a soil sample taken from Rapa Nui, the Polynesian name of Easter Island, rapamycin was first developed as an antifungal agent and later found to have immune suppressant and antitumor properties. Then a 2009 study showed that rapamycin also increased lifespan in mice. Ahmed says that he initially studied the drug to see whether long-term use in transplant patients might damage the immune system. “We really wanted to look at whether rapamycin might be *screwing up* established immunologic memory,” he says. “We had the totally opposite result.”

Ahmed says even though rapamycin has toxic effects in transplant patients who take it for years, clinical trials in adults that combine short-term, low-dose rapamycin treatment with a vaccine make sense. Indeed, he says the U.S. National Institutes of Health seriously considered sponsoring such a trial after his *Nature* study was published 4 years ago, but the idea stalled. “If you use rapamycin for a couple of weeks, it's very unlikely something would happen,” he says. “It's certainly tempting to think about it. And with this study coming out, there'll be more of an impetus.”

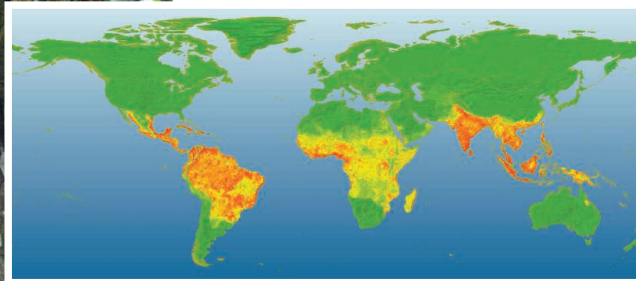
—JON COHEN



Greater reach. Rapamycin broadened antibody binding (colors) on influenza's hemagglutinin protein (gray).

monly infects humans. They then exposed the mice to several different influenza viruses that readily kill the animals, including the H5N1 and H7N9 bird viruses that have jumped into humans and stoked fears of deadly pandemics. Mice treated with rapamycin had significantly higher levels of CD8 T cells against influenza and better survival than control animals not given the immune-suppressing drug.

But the mechanism was not what the researchers expected. “The biggest surprise was that the protection wasn't mediated by the CD8 T cells,” says McGargill, who collaborated with Peter Doherty, an immu-



Jungle fever. Even as dengue spreads (*inset*, redder colors indicate higher infection risk), a new serotype is likely circulating among macaques on Borneo.

TROPICAL MEDICINE

Surprising New Dengue Virus Throws A Spanner in Disease Control Efforts

BANGKOK—Dispatches from the frontlines of the war on dengue are growing more and more dispiriting. In September 2012, pharmaceuticals giant Sanofi Pasteur revealed that a vaccine against the centuries-old tropical malady had stumbled in clinical trials. Meanwhile, dengue's toll is heavier than thought. According to an April report based on modeling, the annual global incidence, close to 390 million, is about three times higher than the number of cases estimated by the World Health Organization (WHO) for 2009. At a meeting here earlier this week, scientists described the latest blow on the battlefield: a fifth dengue virus. The first new serotype discovered in a half century could complicate control efforts, especially the quest for a vaccine that protects against all types simultaneously.

"No other vector-borne disease strikes fear into the hearts of public health workers like dengue," says Lulu Bravo, a pediatrician at the University of the Philippines, Manila. There is no vaccine or drug against dengue, which is spread by *Aedes aegypti* mosquitoes and called "breakbone fever" for the excruciating joint pain in severe cases. Although dengue deaths have dropped thanks to improved patient care, the disease remains the leading cause of hospitalization of children in Thailand, says pediatrician Usa Thisyakorn here at Chulalongkorn University.

Urbanization is fueling dengue's rapid advances. Water collecting in gutters, abandoned tires, and septic tanks, for example, provides convenient breeding grounds for *Aedes* mosquitoes. As investigators reported at the meeting, even as the mosquito's range

expands, possibly thanks to climate change, efforts to control it by eliminating standing water and spraying aren't keeping pace.

Malaysia is a case in point. The nation used to suffer major dengue outbreaks once or twice a decade. "Since 1991, we have had yearly outbreaks," says Mohd Zaki, a vector-borne disease specialist with Malaysia's Ministry of Health. "Dengue is spreading from urban to rural areas and to countries, such as Nepal, where it has not been seen before," adds Samlee Plianbangchang, WHO's Southeast Asia regional director. A 2010 outbreak in Nepal even hit Kathmandu, which sits at an elevation of 1400 meters and was presumed to be inhospitably cool for *Aedes*. "It seems there is some correlation between climate change and dengue moving to higher altitudes," says Basu Dev Pandey, an official with Nepal's health department.

Most dengue infections are mild. But in about 10% of cases, the disease progresses to dengue hemorrhagic fever, in which leaky blood vessels can, in rare instances, lead to death. Infection confers lifetime immunity to a particular serotype, but subsequent infection with a different dengue virus increases the likelihood of severe disease. For that reason, vaccine developers have been striving to concoct a vaccine that protects against the four known serotypes simultaneously. But with limited insight into the complex interactions between the human immune system and the dengue virus, progress has been slow, says infectious disease specialist Scott Halstead, a senior adviser to the Dengue Vaccine Initiative in Seoul. "We have to cope with the fact that we don't understand the science," he says.

The fifth serotype, which almost escaped detection, clouds the picture even more. In 2007, when a dengue outbreak struck Malaysia's Sarawak state, on Borneo, blood and serum samples from a severe case labeled "dengue 4" were collected through a surveillance network set up by Jane Cardosa, a virologist now retired from Universiti Malaysia Sarawak. Later, a researcher found that the samples did not respond to dengue 4 diagnostic tests. Nikos Vasilakis, a virologist at the University of Texas Medical Branch in Galveston, sequenced its entire genome and found that the virus occupies a new branch on the dengue family tree. Vasilakis and his collaborators then determined that antibodies elicited in monkeys and humans by the Malaysian virus differ significantly from those elicited by the other four strains. When injected into monkeys previously stricken with types 1, 2, and 3, the new strain replicated like mad. In monkeys that had recovered from type 4, it replicated poorly, indicating that the monkey immune system saw similarities in the two. "They've done a very good job in characterizing the virus, and it's convincing that it is distinct from the other four," says Thomas Scott, a dengue ecologist at the University of California, Davis.

The public health implications are unclear. Vasilakis says there doesn't seem to be a sustained transmission cycle of serotype 5 in humans, though his team suspects that the virus circulates among nonhuman primates, possibly macaques, on Borneo. For vaccine developers, factoring in a new strain could be a major headache.

Scott says the dengue community is realizing that current control approaches are too simplistic. And vaccines in the pipeline don't appear to provide complete immunity. "Just one tool isn't going to do it," Scott says. "Dengue will be with us for many years," Plianbangchang laments. And as bad as it is, he says, it "could get worse."

—DENNIS NORMILE

CREDITS: KATHRYN HANLEY; (INSET) JANE MESSINA



Varmus's Second Act

Years after steering the National Institutes of Health into a 5-year budget doubling, the Nobel Prize–winning virologist is now helping the cancer institute deal with the aftermath

WITH THE THREAT OF A GOVERNMENT shutdown days away, the mood here in the office of the scientist who leads the nation's efforts to beat cancer is tinged with gloom. National Cancer Institute (NCI) Director Harold Varmus reflects on the current state of biomedical research in the United States, which hasn't seen meaningful budget growth in a decade. "What bothers me most is this sense of hypercompetition," Varmus says. "There's an imbalance between the money available, the work that needs to be done, and the number of people who would need to be supported to make the world feel like a more comfortable place. ... The atmosphere is not as healthy as it ought to be."

No one is in a better position than Varmus to know what ails the U.S. biomedical research community and the agency that sustains it, NCI's parent, the National

Institutes of Health (NIH). His research career began here 45 years ago when the English graduate student-turned-physician joined an NIH lab as a research trainee. In November 1993, not long after he shared the 1989 Nobel Prize in physiology or medicine for using retroviruses to discover cancer genes, he returned to lead NIH.

When Varmus left in 1999, biomedical research was on a roll: Aided by a federal budget surplus, Congress had begun to double NIH's budget, which stood at \$13.7 billion in 1998, over 5 years. Although biomedical research lobbyists came up with the doubling plan, Varmus is credited with winning lawmakers' support with his effective political skills. Now, a decade after the doubling ended in 2003, Varmus is coping with its legacy: what many call the "undoubling."

By July 2010, when Varmus returned to NIH to direct NCI, the funding climate had decisively chilled. For a decade, NIH has received flat or declining budgets. And last year, thanks to the budget cuts known as the sequester, its \$30.6 billion budget dropped another 5.5%. "There was no soft landing," he says. Yet Varmus says his "mantra" is that NCI's \$4.8 billion a year—the largest single slice of NIH's spending—is still a lot of money, and NCI should focus on using it to fund the very best science. He has looked for bloated programs to trim and launched new efforts, such as one that explores the biggest mysteries in cancer and another to find drugs to target a family of mutated proteins that could be an Achilles' heel of many tumors (see sidebar, p. 418).

"You might think that in these times, you would go into a sort of bunker mental-

CREDIT: BLOOMBERG VIA GETTY IMAGES

ity and just hope for a better day. That's not what's happening. He's trying very hard to keep the ship sailing forward in an important new direction," says cancer biologist Tyler Jacks of the Massachusetts Institute of Technology in Cambridge, a former graduate student in Varmus's lab who now chairs NCI's main advisory board. "Harold's chief characteristic is that he's always focused on the science (in the broadest sense) and his judgments reflect that perspective," writes Thomas Kelly, chief of basic research at the Memorial Sloan-Kettering Cancer Center in New York City, in an e-mail. That hasn't stopped grumbling in the community, however, that Varmus isn't doing enough to rescue foundering research labs.

Although his brown hair has thinned more since his last tour at NIH, Varmus looks much the same: tall and slim, with a thick mustache and large, round wire-rimmed glasses. He still favors khaki pants and shirts without a tie. He often bikes his old route 12 miles to work from his apartment in Washington, D.C. But he now considers home to be Manhattan, where he lives with his wife in an apartment on the Upper West Side, not far from his two grown sons. The New York City area is also where he grew up, later went to medical school, and spent 10 years directing Sloan-Kettering between his two tours at NIH. He spends four long workdays in Bethesda—a "compressed week"—then often takes the train to New York City on Friday morning, or flies to his country home near Albany, to work remotely.

In a 1-hour interview in his office, where his staff members are scurrying around planning for the 1 October government shutdown that ended last week, Varmus is candid yet careful, and sometimes prickly. He notes that his middle name is misspelled in the Wikipedia entry someone wrote about him. (It is Elliot, not Elliott.) He alludes, as he often does in public meetings and speeches, to frustration with the government bureaucracy. He says he doesn't particularly want his age mentioned (he's 73) because he's now one of the oldest directors at NIH. But he notes that his "vitality," as he puts it, outstrips his age.

Lure of science

The son of a Long Island doctor and a social worker, Varmus was pulled between following his father's path and pursuing the humanities. He earned a master's degree in English literature, then went to medical school, which whetted his interest in research. He landed at NIH as a so-called "yellow beret," a participant in a program that allowed physicians to work for the Public Health Service

instead of going to Vietnam. There he joined the lab of endocrinologist Ira Pastan, studying bacterial genetics and witnessing the power of early molecular biology. He soon developed a fascination with understanding how some viruses can cause cancer, an interest also driven by the death of his mother from breast cancer.

At the University of California, San Francisco, he formed a 20-year collaboration with Michael Bishop that centered on a retrovirus that causes cancer in chickens. In work that won them the Nobel Prize, they found that such viruses don't cause cancer on their own, but instead carry mutated versions of genes picked up from

"There's an imbalance between the money available, the work that needs to be done, and the number of people who would need to be supported to make the world feel like a more comfortable place."

—HAROLD VARMUS



Political scientist. Varmus's testimony in Congress during the 1990s helped win support for doubling NIH's budget over 5 years.

animals. Those genes control cells' normal growth and development but can go awry—revealing what are now known as proto-oncogenes.

When he accepted President Bill Clinton's offer to direct NIH, Varmus had virtually no administrative or political experience. He quickly learned the ropes of Washington, however, moderating demands from patient groups, fending off conservatives' efforts to ban stem cell research, and winning support from Congress to double NIH's budget. As NIH director, he also proposed a then-radical idea—that biomedical papers should be freely available online—

which led NIH to establish a free archive for full-text papers, PubMed Central, and seeded the open-access journals movement. Making papers and data free would speed the pace of scientific research, Varmus argued.

Years later, when he was preparing to step down after his 10-year stint as Sloan-Kettering president and was helping the Obama transition team look for an NCI director, he accepted a suggestion that he take the job himself, taking a sharp salary cut from \$2.6 million a year to a sum in the mid-\$300,000s. Despite flat budgets, there was "no better time" to head NCI, he later said, because genomics studies were revealing a detailed portrait of cancer cells that

could transform treatment and diagnostics. Compared with heading the entire NIH, "this is much more fun. I don't want to go down to the department [of Health and Human Services] for meetings, I don't want to spend all my time on the Hill" testifying before Congress, he says. He's also said he came back because he has a "profound affection for the NIH."

Varmus now works for NIH Director Francis Collins, who as genome institute director in the 1990s reported to Varmus. The two longtime friends have differed over at least one issue: Collins's swift decision in 2010 to create a new center at NIH aimed at speeding the translation of basic discoveries into treatments. Varmus and National Institute of Allergy and Infectious Diseases Director Anthony Fauci cautioned

that a new body devoted to translational research could overlap with other institutes' existing large translational programs. Years earlier, as NIH director, Varmus had warned that the proliferating number of institutes made NIH less effective; he wanted fewer. Varmus now says that Collins worked "collegially" with him and other directors "to make sure that the definition got right," adding that the National Center for Advancing Translational Sciences is "catalytic" and "works as partners" with other institutes.

Dealing with the pain of tight budgets has been unavoidable. Partly because rising salaries consume so much of NCI's intra-

A Cancer To-Do List

Even as National Cancer Institute (NCI) Director Harold Varmus contends with tight budgets (see main story, p. 416) he has an unusually long list of priorities.

PROVOCATIVE QUESTIONS

Why does obesity increase cancer risk? Why are some cancers easily cured with conventional chemotherapy? Those are among about two dozen little-explored or neglected cancer puzzles that Varmus wants researchers to explore in NCI's Provocative Questions initiative, which has awarded \$35 million in new grants since 2012 and is now taking proposals for a third round of funding.

GLOBAL CANCER

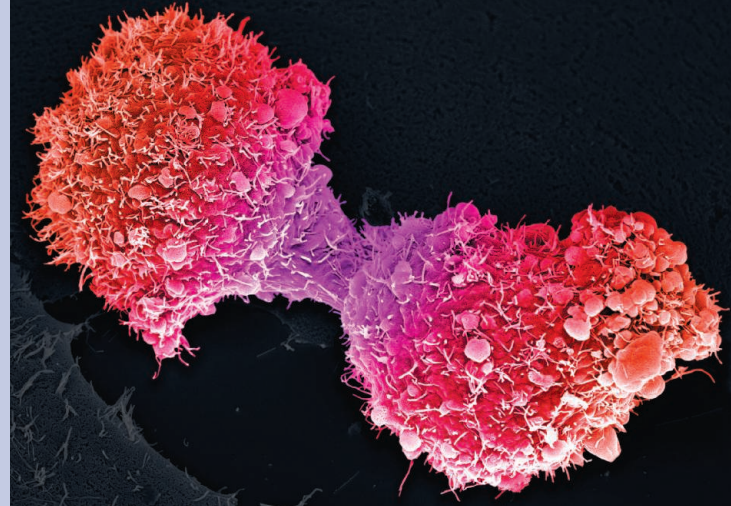
NCI's new Center for Global Health aims to help developing countries create cancer registries and national cancer plans. Another priority is to study the high rates of certain cancers in some countries—gallbladder cancer in Chile, for example.

ONCOGENE INITIATIVE

Varmus is directing \$10 million at NCI's large contract lab in Frederick, Maryland, to finding drugs that target the cell signaling pathway controlled by RAS, a family of oncogenes. Mutations in RAS drive uncontrolled cell growth in one-third of all cancers, so drugs blocking this pathway could help many patients. The initiative is part of an effort to revamp Frederick to focus on large goal-oriented projects, following the model of the Department of Energy's major research labs.

THE CANCER GENOME ATLAS

The Cancer Genome Atlas (TCGA), a \$375 million sequencing project launched with the National Human Genome Research Institute 7 years ago, is winding



Target. Pancreatic cancer cells are usually driven to grow by mutations in RAS genes.

up its analysis of major mutations in 10,000 tumor samples covering about 20 cancer types. One of TCGA's original proponents, Varmus says NCI will soon launch a cloud computing pilot project to support further data mining.

UNCONVENTIONAL TRIAL

NCI plans to genotype the tumors of about 1000 patients who are no longer helped by conventional treatments and match them with an experimental drug aimed at a mutation in the patient's tumor. NCI has assembled a medicine chest of about 30 drugs contributed by a dozen companies. This "unconventional" clinical trial is "one way to dramatically extend our efforts to explore new therapies with genetic tools," Varmus says.

EXCEPTIONAL RESPONDERS

Varmus has asked the research community to comb through their records for rare cases in which a patient's cancer shrank or disappeared in response to a drug that failed to help most patients. The goal is to learn whether specific genetic defects in the patient's tumor can explain such exceptional responses.

mural research budget, the program has closed about 130 of its 300 labs over the past 10 years and reopened only half with new recruits, a "dramatic" change, says intramural Center for Cancer Research Director Robert Wiltout. Coupled with recent federal government-wide restrictions on travel and conferences—NIH cannot even pay for coffee at meetings—morale is very low on the Bethesda NCI campus, researchers say. Wiltout credits Varmus with being "a strong advocate" for allowing staff to continue to travel to conferences.

Looking across NCI, Varmus has found a few places to trim sharply: communications and public relations, such as educational websites and newsletters, and a \$350 million effort to build data-sharing software that was widely recognized as a failure, for example. But in general, he has applied cuts fairly evenly across cancer centers and ongoing grants that have already been funded. His goal is to preserve money for new investigator-initiated grants, the mainstay of cancer research labs. He has also tried to dispel the notion held by some

researchers that NCI now favors translational research, not basic science. "I try to emphasize the fact that there is no one thing that's going to help you get a grant except by doing really interesting science."

To make the grant-review process fairer for proposals that are very similar in qual-

"I try to emphasize the fact that there is no one thing that's going to help you get a grant except by doing really interesting science."

—HAROLD VARMIUS

ity, Varmus added new steps. Proposals that reviewers rank in roughly the top 9% are now funded without further review. But applications that score between 9% and about 25% get a second look from top NCI leaders, who consider program priorities, novelty, and how much funding a researcher already has. This means some grants far below the 9% cutoff receive funding.

Unsustainable

Improving how NCI works won't solve a bigger problem: too many biomedical researchers chasing a dwindling pot of research money. "Our whole system of operating is basically one that is probably not sustainable. That is, the idea that we're going to continue to fund more and more people, train a lot of people, and expand to meet all the opportunities that are there scientifically just does not accord with the current economic situation," Varmus says.

The roots of the problem go back to the NIH doubling, he says. He has no regrets about his support of the doubling itself: "It certainly wasn't a mistake," he says. "But we said at the time, and many people agreed, that this was only going to work well if the ... rapid increase was followed by what we called the soft landing"—budget increases that kept pace with rising costs of doing biomedical research.

Congress did not give NIH those increases, and meanwhile, research institutions built new buildings and labs and hired faculty in the expectation that biomedical research

CREDIT: STANLEY FLEGEL/STANLEY FLEGEL VISUALS UNLIMITED INC.

funding would keep growing. This “was understandable,” Varmus says, but it has created cutthroat competition for grants, with only about one in six proposals now receiving funding, compared with one in three a decade ago. The result, Varmus says, is an “insidious and pervasive problem of people and labs competing for jobs, grants, etc.”

There are no easy solutions, he says, and one inevitable result—academic labs downsizing or closing altogether—is already under way. “We all know that, in a fashion nobody can be comfortable with, there’s a certain amount of attrition going on,” he says.

Meanwhile, a flood of young trainees are seeking scarce academic posts. Varmus says he sees “eye to eye” with Princeton University molecular biologist Shirley Tilghman, who led an NIH-commissioned report last year that urged the agency to shorten the number of training years and channel more young scientists into alternative careers. Like Tilghman, he also wants to encourage labs to rely more on permanent staff scientists rather than exploiting the labor of cheap trainees.

Varmus also wants to address what he calls the “flawed values system” that the competitive atmosphere has spawned. He laments that researchers feel they will win funding only if they publish in top journals such as *Science*, *Nature*, and *Cell*. At NCI, he is pilot-testing a way to change this part of the scientific culture: by revising the “biosketch,” the summary of a researcher’s record that accompanies a grant proposal. Varmus wants to replace a section that now lists major publications with a narrative describing the investigator’s five major accomplishments.

This approach, already used by the Howard Hughes Medical Institute (HHMI), should not only discourage reviewers from assessing their colleagues based only on where they’re published; it will also help researchers on large teams whose names are buried in a list of authors receive the credit they deserve, Varmus says. And he thinks that easing the rush to publish in high-impact journals might help address a much-discussed problem: that many NIH-funded studies have proved difficult to reproduce.

Encouraging openness among scientists could also help, Varmus says. He remains a staunch supporter of free access to research results, and he notes with satisfaction that PubMed Central will soon add new tools so researchers can comment on published papers. This will “promote a higher level of engagement and to-and-fro



Varmus Career Highlights

- 1962 M.A. in English literature from Harvard University
- 1966 M.D. from Columbia University
- 1968–1970 Research trainee in Pastan lab, NIH
- 1970 Postdoctoral fellowship in the lab of Michael Bishop at UCSF leads to collaboration
- 1976 Bishop/Varmus *Nature* paper reporting that cancer-causing gene in Rous sarcoma virus resembles noncancerous gene in normal bird cells
- 1989 Awarded Nobel Prize in physiology or medicine with Bishop (pictured)
- 1993–1999 Director of the National Institutes of Health
- 2000–2010 President of the Memorial Sloan-Kettering Cancer Center
- July 2010–present Director of the National Cancer Institute

on work that’s been published,” he says. He’s pleased that the White House science office is now asking all research agencies to follow NIH’s lead and require that grantees make their papers freely available within a year, although he thinks the directive “could have been stronger,” with a shorter embargo period.

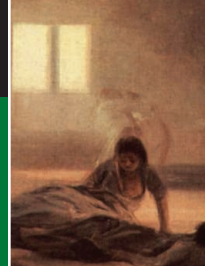
To relieve the constant pressure on investigators to write grant proposals to keep their labs afloat, Varmus is creating a new award at NCI for “outstanding investigators.” It will give highly productive labs support for 7 years, with the possibility of renewal. Although NIH already has a long-term award for established investigators, the winners are chosen largely according to their peer-review scores for a specific project. The new award will go to researchers nominated by their institutions and will be

based on their overall track record. Like HHMI awards, it will go to “people,” not “projects.” Varmus wants NCI to fund hundreds of these awards.

Varmus ticks off other items on his to-do list: controlling cancer in the developing world, for example, by expanding use of the vaccine against human papillomavirus, which causes cervical cancer; getting oncologists to incorporate tumor genetics into treatments; finding better ways to predict whether tumors detected early on will grow or remain small and harmless.

How many of these tasks he will accomplish will depend, he admits, on the next election. “I’m a presidential appointee. Some make it across that boundary, some don’t,” he says. But for now, he says, “I have no plans to leave.”

—JOCELYN KAISER



LETTERS

edited by Jennifer Sills

Ecosystem Services: Accounting Standards

IN THEIR RESEARCH ARTICLE “BRINGING ECOSYSTEM SERVICES INTO ECONOMIC DECISION making: Land use in the United Kingdom” (5 July, p. 45), I. J. Bateman *et al.* demonstrate the importance of considering nonmarket ecosystem services in economic decision-making. It is an excellent example of the potential for national-level spatial analysis of economic and environmental information to inform policy choices.

The drive to connect economic and environmental information mirrors the ongoing developments in environmental-economic accounting. Over the past 6 years, the international statistics community has led work to finalize an international standard—the UN System of Environmental-Economic Accounting (SEEA) (1)—and to place the measurement of ecosystem services and ecosystem condition into a national accounting context (2). The development of these statistical frameworks provides the basis for compiling internationally comparable data sets at a national level on the relationship between the environment and economic activity.

Despite their common motivations, the approaches of Bateman *et al.* and the SEEA differ in the ways that they assign value to ecosystem services. Bateman *et al.* ground their analysis in welfare changes as a consequence of specific policy scenarios. The SEEA approach aims to record the “output” generated by ecosystems, given current uses of ecosystem capital; thus, monetary values represent exchange values consistent with the principles of national accounting.

The SEEA approach provides a way to place welfare-based estimates in a broader context. According to Bateman *et al.*, the maximization of all monetary values leads to an increase of £19,606 million per year with a loss of £448 million in agricultural output [Table 3 in (3)]. This loss equates to just over 2% of current UK agricultural output, and the overall impact of including nonmarket services as a proportion of GDP is an additional 1.3% (3).

However, there are some important differences between the definitions of economic activity used by Bateman *et al.* and standard national accounting, which may limit the interpretation of such comparisons. By integrating estimates of ecosystem services within the framework of accepted economic data, the SEEA approach can provide additional impetus to mainstream these types of studies.

Therefore, in addition to the calls by Bateman *et al.* to ensure the use of additional information on ecosystem services within standard decision-making, we call for investment to improve the quality of the underlying data within a widely accepted and integrated measurement framework such as the SEEA. The availability of quality data is an important precondition to analysis that should not be overlooked.

CARL OBST,^{1*} BRAM EDENS,^{2,3} LARS HEIN⁴

¹Melbourne Sustainable Society Institute, University of Melbourne, Victoria, 3010 Australia. ²National Accounts Department, Statistics Netherlands, 2492 JP, The Hague, Netherlands. ³Faculty of Economics and Business Administration, VU

University Amsterdam, 1081 HV, Amsterdam, Netherlands.

⁴Environmental Systems Analysis Group, Wageningen University, 6700 AA, Wageningen, Netherlands.

*Corresponding author. E-mail: carl_obst@me.com

References

1. United Nations, European Commission, Food and Agriculture Organization of the United Nations, International Monetary Fund, Organisation for Economic Co-operation and Development, World Bank, “System of Environmental-Economic Accounting 2012: Central Framework, pre-publication (white cover)” (2012); http://unstats.un.org/unsd/envaccounting/White_cover.pdf.
2. United Nations, European Commission, Organisation for Economic Co-operation and Development, World Bank, “System of Environmental-Economic Accounting 2012: Experimental Ecosystem Accounting, pre-publication (white cover)” (2013); http://unstats.un.org/unsd/envaccounting/eea_white_cover.pdf.
3. Office for National Statistics, Input-Output Supply and Use Tables—2013 Edition (2013).

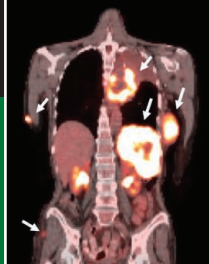
Ecosystem Services:
The Farmers’ Challenge

WE READ THE RESEARCH ARTICLE “BRINGING ecosystem services into economic decision-making: Land use in the United Kingdom” (I. J. Bateman *et al.*, 5 July, p. 45) with interest. The National Farmers’ Union believes that one of the biggest challenges facing farmers and growers in England and Wales will be balancing their commitment to meeting global food demand with their responsibility to reduce environmental impact.

We feel that the economic valuations assigned to ecosystem services should be treated with some caution, as the National Ecosystem Assessment model is far too simplistic. By crudely comparing market prices for agricultural produce against the value of nonmarket goods such as biodiversity, the model disregards the fact that market power may diminish the prices that farmers receive for their output to a level below the value that consumers place on their food. It also fails to account for likely future increases in food prices.

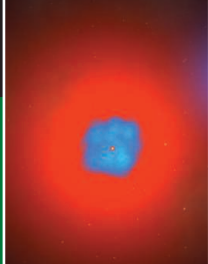
Furthermore, Bateman *et al.*’s analysis suggests that “farming in the United King-





New probes for
PET imaging

429



IBI Prize Essay

436

dom will largely benefit from warmer temperatures.” The United Kingdom’s first Climate Change Risk Assessment (1) also stated that warmer temperatures may present some opportunities to improve yields, with the caveat that reduced water availability may limit these benefits. However, farming is vulnerable to extreme weather events, as we saw last year (2). Recent work by the Government’s Food Research Partnership suggests that the United Kingdom is “potentially at considerable risk from increasing weather extremes, locally for UK production, and globally for UK food prices” (3).

Suggesting that farmers should get paid far more for services they provide to the nation’s ecosystems than the compensation they get for producing food is not only worrying but also misleading. The National Farmers’ Union believes that successful policy-making has its roots in robust scientific evidence, where the assumptions made and uncertainty and limitations associated with the analysis are clear. This is especially critical in the face of a challenge as formidable as climate change, which is likely to present society within the United Kingdom’s borders and across the world with some very difficult decisions.

ANDREA GRAHAM,* HELEN FERRIER,
DIANE MITCHELL, CERIS JONES, PHILIP BICKNELL

Policy Services, Agriculture House, National Farmers’ Union, Stoneleigh, Warwickshire, CV82TZ, UK.

*Corresponding author. E-mail: andrea.graham@nfu.org.uk

References

1. UK Climate Change Risk Assessment: Government Report (The Stationery Office, London, 2012).
2. National Farmers’ Union, “NFU outlook for 2013” (www.nfuonline.com/news/press-centre/nfu-outlook-for-2013/).
3. T. Benton *et al.*, “Severe weather and UK food chain resilience: Detailed Appendix to Synthesis Report” (2012); www.foodsecurity.ac.uk/assets/pdfs/frp-severe-weather-uk-food-chain-resilience.pdf.

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the past 3 months or matters of general interest. Letters are not acknowledged upon receipt. Whether published in full or in part, Letters are subject to editing for clarity and space. Letters submitted, published, or posted elsewhere, in print or online, will be disqualified. To submit a Letter, go to www.submit2science.org.

Ecosystem Services: Nature’s Balance Sheet

IN THE RESEARCH ARTICLE “BRINGING ECOSYSTEM SERVICES INTO ECONOMIC DECISION-MAKING: Land use in the United Kingdom” (5 July, p. 45), I. J. Bateman *et al.* summarize current efforts to assign economic value to ecosystem services. We add three factors that should be taken into account, based on life cycles and contribution of agricultural land use and associated habitats providing food.

First, both income and expenditures should be included in valuation of ecosystem services. Expenditures are a major consideration in land-management decisions alongside income and value of products; recovery of food from ecosystems requires financial and other capital inputs.

Second, using farm gate prices (i.e., the prices when leaving the farm, which are often lower than retail prices paid by consumers) to estimate the contributions of food from agriculture underestimates the product’s value. In 2010, the base year for Bateman *et al.*’s analysis, total UK farm gate income was £4.34 billion, whereas the UK food and drink sector—including manufacture, wholesale, and retail—was valued at £86.2 billion (1). In current ecosystem assessment and economic valuation methodologies, the downstream economic and societal benefits value is not attributed to food production in ecosystem services.

Third, agricultural land management influences ecosystem services beyond food provision. As Bateman points out, in Great Britain, agriculture directly influences about 75% of the land surface through management schemes and crop and livestock production activities. Many other ecosystem services recognized by the Millennium Ecosystem Assessment and UK National Ecosystem Assessment are thus directly and indirectly affected by agricultural land management. Economic valuation and accounting requires increased recognition of, and sensitivity to, interrelationships between services and the contributions made by land management to their availability.

RICHARD ASPINALL^{1,2*} AND PETER GREGORY^{3,4}

¹James Hutton Institute, Craigiebuckler, Aberdeen, AB15

8QH, UK. ²School of Geosciences, University of Aberdeen, King’s College, Aberdeen, AB24 3UE, UK. ³East Malling Research, East Malling, Kent, ME19 6BJ, UK. ⁴School of Agriculture, Policy and Development, University of Reading, Whiteknights, Reading, RG6 6AR, UK.

*Corresponding author. E-mail: richard.aspinall@hutton.ac.uk

Reference

1. Department for Environment, Food and Rural Affairs, Agriculture in the United Kingdom 2011” (2012); www.gov.uk/government/publications/agriculture-in-the-united-kingdom-2011.

Response

C. OBST *ET AL.* PROVIDE A WELCOME OPPORTUNITY to clarify the difference between environmental-economic cost-benefit analyses (such as ours) and environmental accounting exercises [such as the UN-SEEA (1, 2) initiative]. Accounting studies attempt to assess the total value of goods related to ecosystem services in a manner comparable to that used for market-priced goods in national accounts. A decline in the ecosystem services account over time signals a potential need to invest in underlying natural capital. However, such accounts do not indicate the most cost-effective form of that investment. Environmental economic analyses such as ours typically consider changes in value from the status quo that alternative investments provide, and identify those that yield higher value for money. The two approaches are complements rather than substitutes and serve differing but highly compatible elements of the decision-making process.

A. Graham *et al.* criticize our use of “the value of nonmarket goods such as biodiversity.” Although we valued nonmarket greenhouse gas emissions and recreation, we explicitly did not attempt to define biodiversity in terms of economic values; instead, we applied a quantitative constraint prohibiting the degradation of biodiversity within our scenario analyses and examined the costs this would entail, finding them to be minor relative to other values. Graham *et al.*’s critique that we should not compare nonmarket values for ecosystem services with the market price of agricultural output ignores the fact that, as stated, we are conducting an economic analysis of marginal changes from the status quo and not attempting to assess the total value of food. In such assessments of changes, the use of market prices is standard (3). Indeed, there is an argument (3) that such analyses should subtract subsidies (including income support), which would reduce agricultural values (4). Graham *et al.* also question the possible increase in yields attributable to climate change. We do believe

that UK farming will generally benefit from warmer temperatures but caution that [as detailed in (5)] within areas of lower rainfall, increased drought could potentially reduce or even reverse these gains.

We agree with R. Aspinall and P. Gregory that it would be better to consider net profits rather than farm gate prices, although again this would have reduced estimates of agricultural values. The need to link land use to its ecosystem service impacts favored our use of Agricultural Census (6) data, which omits profits. We are currently addressing this through a link to the Farm Business Survey (7) database. However, we disagree with the authors' contention that we should have included the added-value of post-farm food processing. Aside from the fact that the UK food processing industry is a major importer of non-UK produce, such an approach would be analogous to valuing timber at the price of fine furniture. It is the raw material value that is relevant here. Similarly, our analysis explicitly links agricultural land use to its impacts on the ecosystem service considered.

IAN J. BATEMAN,^{1*} AMII R. HARWOOD,¹
GEORGINA M. MACE,² ROBERT T. WATSON,³

DAVID J. ABSON,^{4,5} BARNABY ANDREWS,¹
AMY BINNER,¹ ANDREW CROWE,⁶ BRETT H. DAY,¹
STEVE DUGDALE,¹ CARLO FEZZI,¹ JO FODEN,⁷
DAVID HADLEY,^{1,8} ROY HAINES-YOUNG,⁹
MARK HULME,¹⁰ ANDREAS KONTOLEON,¹¹
ANDREW A. LOVETT,¹ PAUL MUNDAY,¹
UNAI PASCUAL,^{11,12} JAMES PATERSON,¹³
GRISCHA PERINO,^{1,14} ANTARA SEN,¹
GAVIN SIRIWARDENA,¹⁰ DAAN VAN SOEST,^{15,16}
METTE TERMANSEN¹⁷

¹Centre for Social and Economic Research on the Global Environment, School of Environmental Sciences, University of East Anglia, Norwich Research Park, Norwich, NR4 7TJ, UK.

²Department of Genetics, Ecology and Environment, University College London, London, WC1E 6BT, UK. ³Department for Environment, Food and Rural Affairs (Defra), London, UK.

⁴FuturES Research Center, Leuphana Universität, D-21335, Lüneburg, Germany. ⁵School of Earth and Environment, University of Leeds, LS2 9JT, UK. ⁶The Food and Environment Research Agency, Sand Hutton, York, YO41 1LZ, UK. ⁷Centre for Environment, Fisheries and Aquaculture Science (Cefas), Lowestoft, NR33 0HT, UK. ⁸UNE Business School, University of New England, Armidale, NSW 2350, Australia. ⁹Centre for Environmental Management, School of Geography, University of Nottingham, Nottingham, NG7 2RD, UK. ¹⁰British Trust for Ornithology, Thetford, IP24 2PU, UK. ¹¹Department of Land Economy, University of Cambridge, Cambridge, CB2 1TN, UK. ¹²Basque Centre for Climate Change (BC3) and IKERBASQUE, Basque Foundation for Science, 48011, Bilbao, Spain. ¹³School of Geosciences, University of Edinburgh, Edinburgh, EH9 3JW, UK. ¹⁴School of Business, Economics and Social Sciences, University of Hamburg, 20354,

Hamburg, Germany. ¹⁵Department of Spatial Economics and IVM, VU University, 1081 HV, Amsterdam, Netherlands. ¹⁶Department of Economics, Tilburg University, 5000 LE, Tilburg, Netherlands. ¹⁷Department of Environmental Science, Aarhus University, DK-4000, Roskilde, Denmark.

*Corresponding author. E-mail: i.bateman@uea.ac.uk

References

1. United Nations, European Commission, Food and Agriculture Organization of the United Nations, International Monetary Fund, Organisation for Economic Co-operation and Development, World Bank, "System of Environmental-Economic Accounting 2012: Central Framework, pre-publication (white cover)" (2012); http://unstats.un.org/unsd/envaccounting/White_cover.pdf.
2. United Nations, European Commission, Organisation for Economic Co-operation and Development, World Bank, "System of Environmental-Economic Accounting 2012: Experimental Ecosystem Accounting, pre-publication (white cover)" (2013); http://unstats.un.org/unsd/envaccounting/eea_white_cover.pdf.
3. H. M. Treasury, *The Green Book: Appraisal and Evaluation in Central Government* (The Stationery Office, London, 2003).
4. I. J. Bateman, A. A. Lovett, J. S. Brainard, *Applied Environmental Economics: A GIS Approach to Cost-Benefit Analysis* (Cambridge Univ. Press, Cambridge, 2003).
5. C. Fezzi et al., *Env. Res. Econ.*, 10.1007/s10640-013-9663-x (2013).
6. Department for Environment, Food and Rural Affairs, *June Agricultural Census* (Edina, Manchester, UK, 2010).
7. Rural Business Research, "Farm Business Survey" (2013); www.fbpartnership.co.uk.

APPLIED PHYSICS

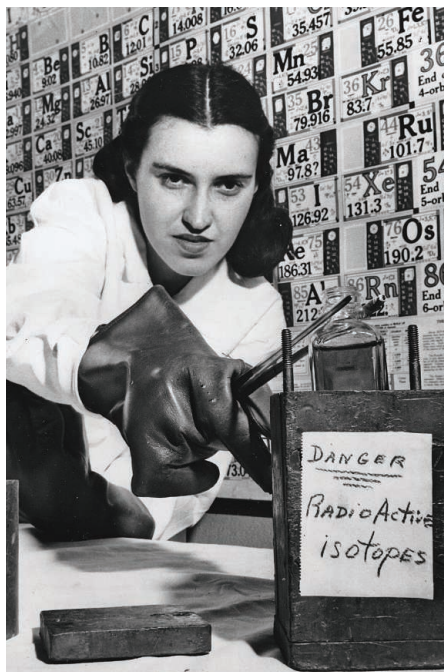
Atomic Ironies

Joanna Radin

Less than a year after the end of World War II, *Science* published the first catalog of United States-produced radioactive isotopes available for order by scientists around the world (1). The catalog was an expression of hope that radiation would transcend associations with the violence of Hiroshima and Nagasaki to become an instrument of peace and prosperity.

Radioisotopes, first produced in cyclotrons in the 1930s, quickly became valued for their potential to serve as medical therapies and for their ability to trace the movement of molecules through living systems. They were hailed as “a new mode of perception.” The energy radioisotopes emitted as they decayed allowed them to be used to illuminate previously invisible transformations in metabolic processes. As the Cold War set in, and nuclear reactors enabled them to be produced en masse, radioisotopes also became instruments of diplomacy. The U.S. Atomic Energy Commission (AEC) found itself mired in controversy about whether or not the government should be providing foreign scientists with radioactivity. Some argued that to do so would be an unacceptable breach of national security. Others believed that sharing such valuable research materials would serve as “emblems of humanitarianism, against the backdrop of an escalating nuclear arms race.”

Focusing on the period between 1945 and 1965, historian Angela Creager (Princeton University) uses radioisotopes to trace the “consequences of the ‘physicists’ war’ for postwar biology and medicine.” With chapters that follow the production, distribution, and uses of these deceptively quotidian research materials, *Life Atomic* provides a striking portrait of the emergence of Cold War science. The book contributes to a growing historical literature that has begun to reconfigure our understanding of the period and its enduring legacies [e.g., (2–4)]. The brilliant light Creager casts on the small pieces of large-scale science shines into the present, where



Preparing an “atomic cocktail.” Nuclear physicist Rosalyn Yalow at the Bronx Veterans Administration Hospital.

research objects such as DNA and cell lines circulate through technical networks in ways scientists, citizens, and policy-makers have yet to fully appreciate.

Creager is deeply interested in exposing the ironies that resulted from the way radioisotopes blurred boundaries between military and civilian uses of radioactivity. The therapeutic potential of radioisotopes was a cornerstone of the AEC’s efforts to promote nuclear energy as a dual-use technology, one with overlapping applications in welfare and warfare. As they became tools of international trade, politics, experimental biology, medicine, and ecology, these by-products of the bomb paid simultaneous dividends in the study of life as well as its destruction. Enthusiasm about civilian applications of radiation was used to justify enduring investments in militaristic ones, “even as the resulting scientific knowledge [about risks] corroded this optimistic view.”

Nowhere is this irony more evident than

in attention to human experimentation with radioisotopes (5). American scientists were secretly injecting civilian volunteers with plutonium in military experiments at the same time that members of the American Medical Association were participating in the prosecution of Nazi scientists at Nuremberg. This prompted the first documented use of the term “informed consent” to militate against public backlash should the government’s plutonium experiments become public. Importantly, however, the AEC’s efforts to regulate military experiments with atomic energy did not extend to the biomedical consumers who purchased radioisotopes from the agency for use as either cancer therapies or diagnostic tools.

Medical applications of radioisotopes were not necessarily harmful—many emitted less radiation than normal exposure to sunlight—but Creager’s skill in following the tracers shows the differential benefits of scientists’ and subjects’ intimate encounters with radioactivity. Take the work of nuclear physicist Rosalyn Yalow, who—along with internist Solomon Berson—helped establish the Radioisotope Unit at the Veterans Administration Hospital in the Bronx, New York. Starting in 1950, they served “atomic cocktails” of iodine-131 to veterans with the ultimate goal of refining the use of radioisotopes for investigations of metabolic disorders such as diabetes. In their convalescence, veterans served on the frontlines of biomedicine as unwitting “experimental vessels” in the development of an influential technique known as radioimmunoassay, which helped Yalow earn the Nobel Prize in 1977. (Berson was deceased and thus ineligible.)

As radioisotopes revealed the temporality of living processes, they left residues that reveal profound alterations in bodies, environments, geopolitical relationships, and science itself. These traces are the historical by-products of Americans’ uneasy and still unfolding embrace of life atomic. Creager’s deft attention to the ironies that have accompanied efforts to harness the atom is history of science at its best: a crystal clear portrait of just how untidy the impacts of science can be.

References

1. “Availability of radioactive isotopes,” *Science* **103**, 697 (1946).
2. J. Krige, *American Hegemony and the Postwar Reconstruction of Europe* (MIT Press, Cambridge, MA, 2006).
3. P. Westwick, *The National Labs: Science in an American System* (Harvard Univ. Press, Cambridge, MA, 2006).
4. J. D. Hamblin, *Arming Mother Nature: The Birth of Catastrophic Environmentalism* (Oxford Univ. Press, Oxford, 2013).
5. M. J. Santemas, *Dynamis* **29**, 337 (2009).

Life Atomic
A History of
Radioisotopes in
Science and Medicine

by **Angela N. H. Creager**
University of Chicago Press,
Chicago, 2013. 505 pp. \$45,
£31.50. ISBN 9780226017808.
Synthesis.

The reviewer is at the Program for the History of Science and Medicine, Yale School of Medicine, New Haven, CT 06520, USA. E-mail: joanna.radin@yale.edu

ECOLOGY

36 Responses on Invasive Species

Montserrat Vilà

Humans have introduced species to areas where they could not disperse naturally. Some of these nonnative (also termed alien or exotic) species successfully spread in the wild, becoming invasive species. Biological invasions, omnipresent around the world, affect biodiversity, ecosystem services, and human well-being. Well-known examples include killer alga (*Caulerpa taxifolia*), cane toad (*Rhinella marina*), cheatgrass (*Bromus tectorum*), golden apple snail (*Pomacea canaliculata*), tiger mosquito (*Aedes albopictus*), and muskrat (*Ondatra zibethicus*).

Invasive Species: What Everyone Needs to Know addresses 36 interdisciplinary questions on biological invasions. Ecologist Daniel Simberloff (University of Tennessee) enriches his responses with more than 500 examples from a broad array of taxa, ecosystem types, and geographic regions. He notes the major progress achieved since the early 1990s, when the Rio Convention on Biological Diversity included the goal to “prevent the introduction of, control or eradicate those alien species which threaten ecosystems, habitats or species.”

Nonnative species arrive via diverse pathways. Some are deliberately released into the wild—for example, for sport hunting or fishing, to feed livestock, and even for conservation and restoration purposes (such as “enriching” the biota of degraded lands). Pets and many ornamental plants escape from homes and yards. Invasive species may be introduced unintentionally in ballast water, as hitchhikers on other nonnatives, or in commodities. Increased travel and trade, high demand for new commodities, construction of major infrastructures, and opening of new commercial routes challenge the control of nonnative species’ entrances and exacerbate their impacts. Interactions among two or more invasives can intensify their impacts on native species, communities, and ecosystems, producing what Simberloff terms an “invasional meltdown.”

The reviewer is at the Estación Biológica de Doñana (EBD-CSIC), 41092 Sevilla, Spain. E-mail: montse.vila@ebd.csic.es



Revised invasive. Cheatgrass (drooping brome or downy brome) has replaced native flora in grasslands throughout western North America.

In the past two decades, invasion ecologists have devoted much research to identifying predictors of which species will become invasive and where. This includes investigations of species traits that confer high fitness and of biotic interactions that limit or facilitate their establishment (often in environmental conditions not faced in their home ranges). Surveying such work, the book demonstrates common, major ecological patterns involving invaders. Yet, as Simberloff notes, “Many rules in ecology and evolution are not rules in the sense of the laws

of thermodynamics but simply statements of patterns that are more or less dominant, although there are always exceptions.”

Research on biological invasions has also greatly increased our understanding of species evolution and ecology at all levels of ecological complexity (1, 2). This is a major reason why scientists are attracted to the topic. In fact, biological invasions provide giant experiments for addressing questions such as the mechanisms of fast evolution, the persistence of small populations, the rules of species assemblages, and the effects of a single species on ecosystem functioning. Simberloff’s account shows that research on biological invasions is well integrated into mainstream ecology or evolutionary biology.

In the realm of applications, the book provides compelling information for improving

conservation of native species and ecosystems threatened by invasive species. Management of biological invasions basically comprises prevention, early detection, and eradication. Simberloff devotes a third of the book to aspects of effective management of invasive species. He describes not only technological improvements but also policy options, cultural perceptions, citizen-science initiatives, and controversies surrounding biological invasions.

“In a policy and management context, it makes little sense to consider all introduced species or even all invasive introduced species in the aggregate.” Thus, we need to provide managers risk assessment tools for modeling the potential distribution of nonnative species and protocols for scoring species based on their likelihood of invading and disrupting. Despite rapid

advances, much work still needs to be done, especially on screening the risks of accidental introductions. This requires a thorough exploration of the links among pathways of introduction, invasion success, and impact (3). Undoubtedly, these analyses must integrate biology with socioeconomic information such as trading trends.

As many researchers realize (4), the management of nonnative species can generate considerable controversy. Major disputes arise from judgments that some actions against introduced species are xenophobic, some introduced species are beneficial, and efforts to control some invasives are futile. Noting positions of conservationists, philosophers, lawyers, and even politicians, Simberloff discusses these controversies in depth.

Written for nonexpert but educated readers, *Invasive Species* will reward those who demand well-documented information without requiring the scientific details. By extending his wide-ranging survey of biological invasions beyond their biology, Simberloff acknowledges the crucial human dimensions of invasive species (5).

References

1. R. M. Callaway, J. L. Maron, *Trends Ecol. Evol.* **21**, 369 (2006).
2. D. F. Sax et al., *Trends Ecol. Evol.* **22**, 465 (2007).
3. B. Leung et al., *Ecol. Lett.* **15**, 1475 (2012).
4. D. Simberloff et al., *Trends Ecol. Evol.* **28**, 58 (2012).
5. J. A. McNeely, Ed., *The Great Reshuffling: Human Dimensions of Alien Species* (IUCN, Cambridge, 2001); <http://data.iucn.org/dbtw-wpd/edocs/2001-002.pdf>.

10.1126/science.1245174

CONSERVATION

Biodiversity Risks from Fossil Fuel Extraction

The overlapping of biodiverse areas and fossil fuel reserves indicates high-risk regions.

N. Butt,^{1*} H. L. Beyer,¹ J. R. Bennett,¹ D. Biggs,¹ R. Maggini,¹ M. Mills,² A. R. Renwick,¹ L. M. Seabrook,^{1,3} H. P. Possingham¹

Despite a global political commitment to reduce biodiversity loss by 2010 through the 2002 Convention on Biological Diversity, declines are accelerating and threats are increasing (1). Major threats to biodiversity are habitat loss, invasion by exotic species and pathogens, and climate change, all principally driven by human activities. Although fossil fuel (FF) extraction has traditionally been seen as a temporary and spatially limited perturbation to ecosystems (2), even local or limited biodiversity loss can have large cascade effects on ecosystem function and productivity. We explore

the overlap between regions of high marine and terrestrial biodiversity and FF reserves to identify regions at particular risk of ecosystem destruction and biodiversity loss from exposure to FF extraction.

Consumption of FF (oil, natural gas, and coal) grew from 26,200 million barrels of oil equivalent (MBOE) in 1965 to 80,300 MBOE in 2012 (3). By 2035, oil demand is projected to increase by over 30%, natural gas by 53%, and coal by 50% (4). It is often assumed that legally mandated restoration after extraction (which includes drilling and all forms of mining) will return an area to close to its pre-disturbance state (2). Extraction activities have therefore been considered trivial disruptors of natural systems in comparison with other human activities, such as agricultural land clearing (5).

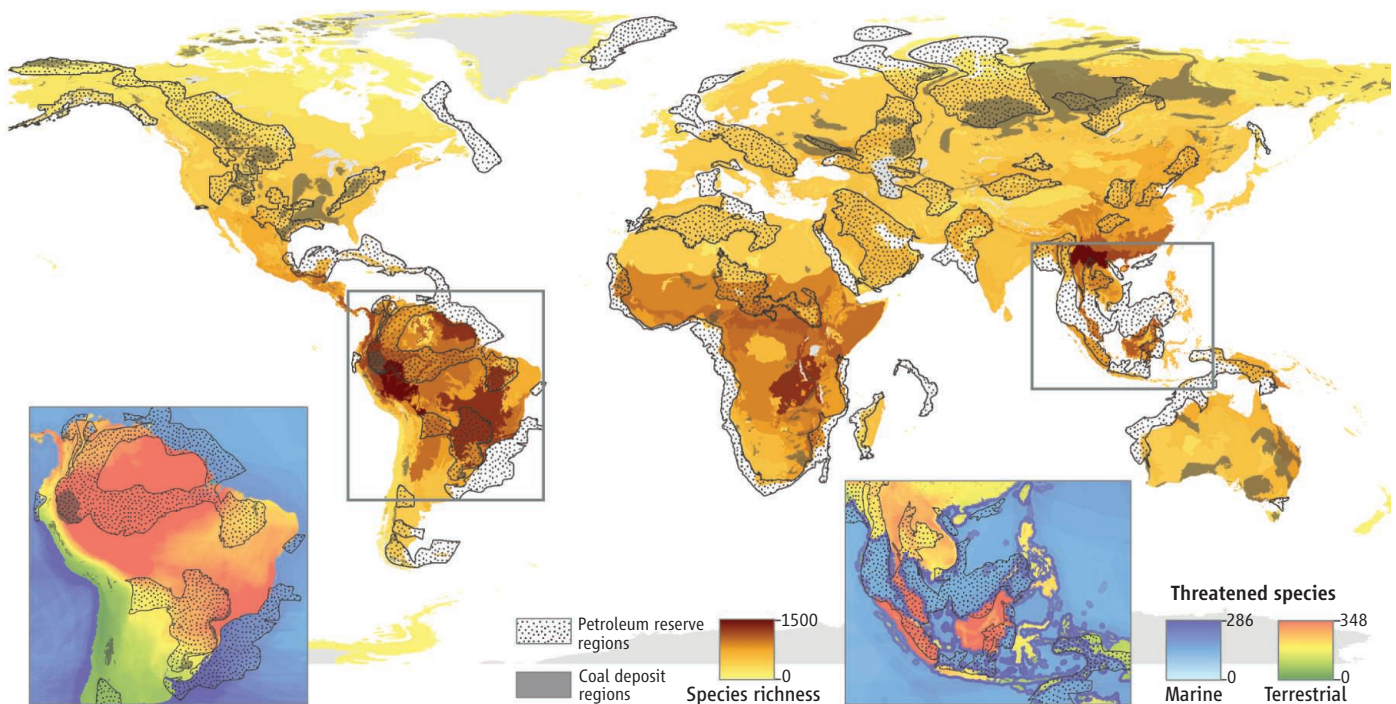
Ecosystem disturbance and degradation resulting from direct or indirect effects of extraction, however, have profound and

enduring impacts on systems at wider spatial scales (6). Direct effects include local habitat destruction and fragmentation, visual and noise disturbance, and pollution (7). Indirect effects can extend many kilometers from the extraction source and include human expansion into previously wild areas, introduction of invasive species and pathogens, soil erosion, water pollution, and illegal hunting (7). Combined, these factors lead to population declines and changes in community composition (8). Gas and oil transportation can also be environmentally damaging, particularly in countries with weak governance, and can lead to deforestation, water contamination, and soil erosion (9). Spills in marine environments can have severe environmental impacts over wide areas (10). However, the main impact of FF extraction on biodiversity may be through facilitating other threats, such as deforestation driven by road construction.

In the future, FF will be increasingly

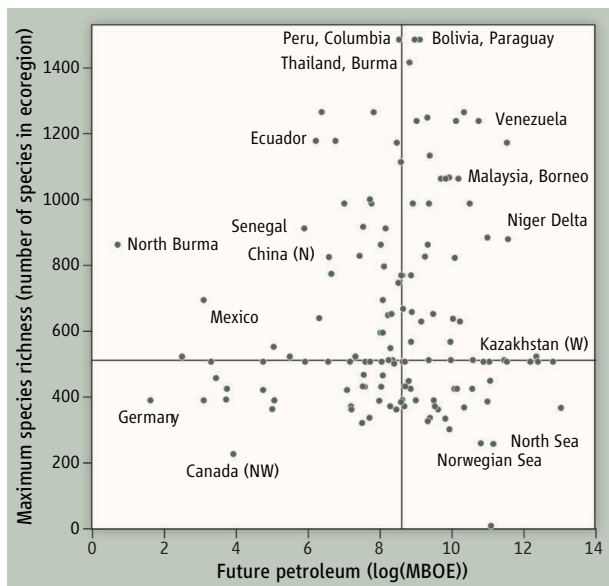
¹Australian Research Council Centre of Excellence for Environmental Decisions, School of Biological Sciences, The University of Queensland, St. Lucia, Queensland, 4072, Australia. ²Global Change Institute, The University of Queensland, St. Lucia, 4072, Australia. ³School of Geography, Planning and Environmental Management, The University of Queensland, St. Lucia, 4072, Australia.

*Corresponding author. n.butt@uq.edu.au



Distribution of FF reserves and species biodiversity. Large map reflects terrestrial species richness (number of species per ecoregion). (Insets) Two regions where many threatened terrestrial and marine species may be affected by FF extraction (background map depicts point estimate counts of threatened spe-

cies ranges at the center of each 0.1° grid cell). Limitations in available data on FF reserves and extraction (e.g., coal reserves in Europe and India) suggest our analyses may underestimate the extent of overlap between FF reserves and regions of high biodiversity. See SM for details.



Ecoregional species richness and petroleum reserves. Quadrants determined by median values for petroleum and species richness. Examples of ecoregions within our identified areas of biodiversity concern include Bolivia, Venezuela, Malaysia, and Borneo. See SM for details.

extracted from more remote and previously undisturbed areas. Unconventional sources, such as coal seam gas and shale oil, will threaten currently undeveloped regions that are biodiverse and represent important centers of endemism (8). Furthermore, the corporations of the FF extraction industry are economically and politically powerful, whereas many countries in areas of high biodiversity risk under FF exploration are characterized by weak governance and poor implementation of environmental regulations.

Areas at Greatest Risk

We suggest that northern South America and the western Pacific Ocean are at particular risk [(see the first figure); see supplementary materials (SM)]. The Western Amazon is one of the most biodiverse regions of the planet and contains large reserves of oil and gas (11). Potential impacts from FF extraction in this region include deforestation, contamination, and wastewater discharge. Increased accessibility to previously remote areas via oil industry roads and pipeline routes is one of the primary causes of habitat fragmentation and facilitates further logging, hunting, and deforestation (11).

The Coral Triangle in the western Pacific Ocean is one of the most biodiverse marine areas of the world, containing 76% of the world's coral species and 37% of the world's coral reef fish species (12). In Papua New Guinea, terrestrial FF development will likely be accompanied by maritime extraction and transport of FF, posing increasing

risk to globally important mangroves (13) and possibly compounding existing threats to coral reefs (14). An oil well failure analogous to the Deepwater Horizon spill or a tanker spill comparable to that of the Exxon Valdez could have devastating consequences for biodiversity in the Gulf of Papua.

Utilizing available data, we explored the spatial coincidence of terrestrial species richness with petroleum reserves (see the second figure). Extraction and processing costs and the size and quality of reserves may strongly influence the prioritization of different regions for exploitation. In principle, however, jurisdictions with large reserves and high biodiversity (e.g., Bolivia, Venezuela, Malaysia, and Borneo) are of particular concern. Developments in these countries are likely to cover a greater spatial extent and so pose threats to numerous species. Regions with large petroleum deposits but low species richness, such as the North Sea, are expected to experience ecosystem degradation, but as species richness is low, the net impact on biodiversity may be relatively small.

Policy Implications and Solutions

Our results highlight opportunities where international FF extraction corporations and conservation organizations can have important impacts on biodiversity protection. We propose that industry regulation, monitoring, and conservation should be targeted where FF reserves and regions of high biodiversity overlap. We suggest that, in general, regions or countries in the high-risk areas with weak governance and low levels of environmental protection may not attract or allow international scrutiny, and so environmental damage caused in these areas may remain both undetected and unaddressed (15). There is a risk, therefore, of noncompliance with the best environmental and safety practices. By contrast, where high environmental standards are enforced, such as the construction of the 3150-km Gasbol pipeline in Brazil and Bolivia, impacts on biodiversity can be minimized (16).

Monitoring biodiversity and the environment is crucial for effective implementation of both industry regulations and conservation management. It is critical that environmen-

tal organizations play an active role in ensuring that FF extraction takes place according to best practices and, ideally, avoids areas of high biodiversity and that trade-offs between biodiversity and development are assessed critically (17). Greater international collaboration between governments, FF extraction corporations, research bodies, and nongovernmental organizations is needed.

With increasing global demand for energy, the location, extent, and methods of extraction are changing rapidly, but the effect on biodiversity of these changes is largely unknown. We speculate—on the basis of the best available, but incomplete, data—that northern South America and the western Pacific Ocean are two critical regions at risk from increasing FF development. Thus far, there has been little research into potential mitigation measures (8). Recognition of the direct and indirect threats to biodiversity from FF extraction in these regions, and of their complex interactions, is essential in the establishment of suitable norms and processes that can guide development to minimize environmental damage.

References and Notes

1. S. H. M. Butchart *et al.*, *Science* **328**, 1164 (2010).
2. M. C. Ruiz-Jaen, T. M. Aide, *Restor. Ecol.* **13**, 569 (2005).
3. BP, Statistical Review of World Energy (BP, London, 2013); www.bp.com/en/global/corporate/about-bp/statistical-review-of-world-energy-2013.html.
4. Institute for Energy Research, Energy Information Association Forecast, www.instituteofenergyresearch.org/2011/09/22/eia-forecast-world-energy-led-by-china-to-grow-53-percent-by-2035/.
5. R. H. Cristescu, C. Frère, P. B. Banks, *Biol. Conserv.* **149**, 60 (2012).
6. IUCN, ICMM, *Integrating Mining and Biodiversity Conservation: Case Studies from Around the World* (IUCN, Gland, Cambridge, ICMM, London, 2004).
7. F. G. Bell, L. J. Donnelly, *Mining and its Impact on the Environment* (CRC Press, Boca Raton, FL, 2006).
8. J. M. Northrup, G. Wittenmyer, *Ecol. Lett.* **16**, 112 (2013).
9. D. O'Rourke, S. Connolly, *Annu. Rev. Environ. Resour.* **28**, 587 (2003).
10. P. F. Kingston, *Spill Sci. Technol. Bull.* **7**, 53 (2002).
11. M. Finer, C. N. Jenkins, S. L. Pimm, B. Keane, C. Ross, *PLoS ONE* **3**, e2932 (2008).
12. World Wildlife Fund, <http://www.wwf.panda.org>.
13. M. Lewis, R. Pryor, L. Wilking, *Environ. Pollut.* **159**, 2328 (2011).
14. H. M. Guzmán, K. A. Burns, J. B. C. Jackson, *Mar. Ecol. Prog. Ser.* **105**, 231 (1994).
15. P. G. Fredriksson, J. Svensson, *J. Public Econ.* **87**, 1383 (2003).
16. J. D. Quintero, A. Mathur, *Conserv. Biol.* **25**, 1121 (2011).
17. P. M. Pedroni *et al.*, *J. Appl. Ecol.* **50**, 539 (2013).

Acknowledgments: This research was conducted with support from the Australian Research Council. We are grateful for comments from P. Baruya (International Energy Agency) and C. Aldridge (U.S. Geological Survey).

Supplementary Materials

www.sciencemag.org/content/342/6158/425/suppl/DC1

10.1126/science.1237261

ECONOMICS

Women, Fertility, and the Rise of Modern Capitalism

Alberto Alesina

The wealth of a nation is measured by its income per capita (total economic output/total population). The latter increases only if the numerator grows faster than the denominator. For most of human history, when the numerator grew, the denominator followed suit, leaving the ratio stable. Income per capita increased only for relatively brief periods and in specific places, and often fell, even dramatically. Two

revolutions that occurred in Europe broke this state of immobility: the Malthusian revolution, spurred by the Black Death (or plague) in the Middle Ages, which slowed the growth of the denominator, and the industrial revolution, which sharply increased the growth of the numerator. The former event has been considered a precondition for the latter because it allowed per capita income to rise well above the subsistence level. This created a demand for goods and the accumulation of wealth and human capital. The outcome was a revolution in technologies that pulled away from traditional agriculture. Thus, this complex interaction of diverse factors spurred the birth of the modern economic era. This includes the impact of women in the workforce, as suggested in a recent study by Voigtländer and Voth (1).

In the late 1700s, British scholar Thomas Robert Malthus argued that population growth is continuously held in check by the resources available to sustain it—a predicament in the sense that this hinders social progress. Indeed, prior to the industrial revolution, income per capita (in terms of food, clothing, and housing, for example) did not generally change. Average living standards in Europe were roughly constant. Spread of the Black Death across Europe from 1348 to 1350 had an obvious direct effect on population size—more than one-third of the European popula-



tion died from the pandemic. But it also had an important indirect effect (1). In the aftermath of the plague, labor became a scarce resource, whereas land became abundant. Technology shifted to land-intensive practices. The scarcity of labor sparked an increase in wages, but it also created many employment opportunities for women. For instance, land-intensive pastoral activities increased their share in agricultural output and allowed the participation of women in the labor force. Young women and girls were needed in the field. As a result, the age of marriage increased and fertility decreased, slowing the recovery of a European population already decimated by the Black Death. The average marriage age of women increased from the late teens before the Black Death to the early mid-20's in the centuries that followed.

Indeed, agricultural technologies appropriate for women and children not only made women a valuable asset, but children as well, creating an incentive for an increase in fertility. Evidence from a different context is consistent with a link between agricultural technologies and fertility. Ethnic groups that traditionally used the plow, which required upper-body strength, had a strict division of labor, with men working in the fields and women at home. In ethnic groups that favored shifting cultivations, women and children worked in the field alongside men (2). Because children were valuable as a labor force, fertility was higher within ethnic groups that did not adopt the plow (3). However, the higher wages gen-

How did the Black Plague change work and family opportunities for women?

Job opportunities. More than one-third of Europe died from the plague in the mid-14th century. As labor became scarce, women joined the workforce and married later, and fertility decreased.

erated by land abundance and labor scarcity could have had an income effect on fertility. That is, on one hand, women had less time for maternity because they worked and tended to marry later. However, a

higher income might have increased the possibility of having more children and improving the quality of their care. These factors may have played out differently in different parts of Europe and the world. Why?

One possibility is that this Malthusian revolution sparked a divergence in the growth of income in Europe and other parts of the world even before the industrial revolution (1). But even within Europe, there was an important difference. The Black Death ravaged most of Europe, but after the plague epidemic, fertility did not decrease much in Southern and Eastern Europe, whose populations returned relatively soon to their levels before the pandemic. Instead, fertility increased substantially in Northwestern Europe. Not surprisingly, this part of Europe led the spectacular rise of modern capitalism.

This observation suggests that the Black Death alone, even accounting for its indirect effect on agriculture, marriage age of women, and fertility, cannot be the only explanation for the industrial revolution and the birth of capitalism. There must be other reasons for the critical divergence in the growth of income in different parts of Europe in the centuries after the Black Death. One possible factor is the Protestant revolution.

Lutheran doctrine (promoted by German reformer Martin Luther) influenced the accumulation of human capital in regions that adopted Protestantism (4). Such regions insisted on the requirement of learning how to read and master the Bible, which was trans-

Department of Economics, Harvard University, Cambridge, MA 02138, USA. E-mail: aalesina@harvard.edu

lated into German for this reason. This may be considered a variation of, or possibly an addition to, the Weberian theory on the religious origin of modern capitalism. The German sociologist Max Weber argued that the teaching of hard work and frugality, and especially the view that economic success in life was a sign of being chosen for a next life, spurred entrepreneurship. Perhaps it was not the religious teaching and moral values of Protestantism that created an economic growth impetus, but rather the latter's empha-

sis on the accumulation of human capital, of men and women (4).

The Malthusian revolution plus the increased participation of women in agriculture and reduction in fertility—coupled, in parts of Europe, with a new emphasis on the accumulation of human capital and possibly some religious fervor—would constitute the perfect catalyst for the birth of capitalism and the rapid social and economic growth that followed. It was the complex interaction of a plague, cultural values, accumulation of

human capital, and development of new technologies that created the spark that ignited the modern economic system.

References

1. N. Voigtländer, H.-J. Voth, *Am. Econ. Rev.* **103**, 79 (2013).
2. A. Alesina *et al.*, *Q. J. Econ.* **128**, 469 (2013).
3. A. Alesina, P. Giuliano, N. Nunn, *Am. Econ. Rev. Pap. Proc.* **101**, 499 (2011).
4. S. O. Becker, L. Wessman, *Q. J. Econ.* **124**, 531 (2009).

10.1126/science.1246228

EVOLUTION

Natural Selection and Pain Meet at a Sodium Channel

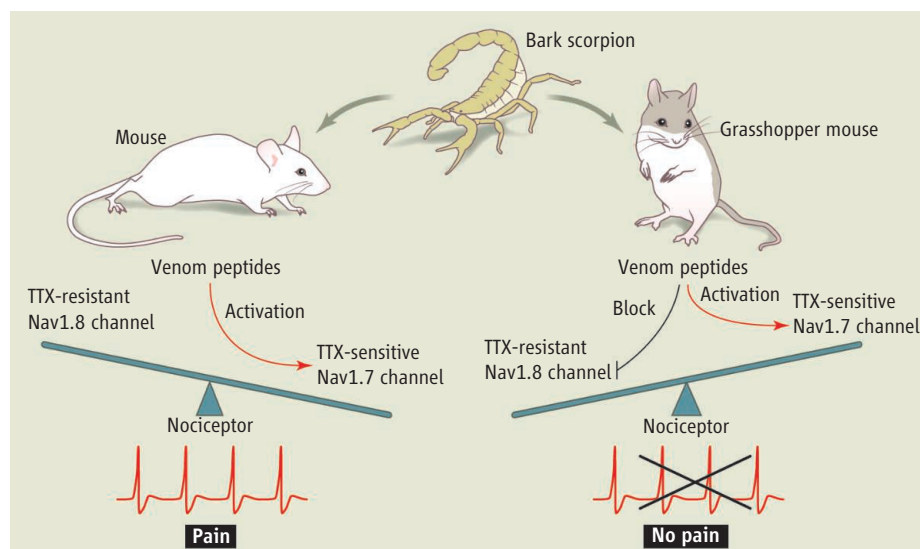
Gary R. Lewin

In the animal kingdom, extreme conditions drive adaptive change to enable species to live in and exploit challenging environments. Nociceptors—sensory fibers activated by noxious stimuli to signal pain—enable animals to detect and avoid potentially harmful stimuli, presumably because such stimuli lead to the experience of pain. Variation in pain sensitivity has not traditionally been considered as a trait that is selected for, or against, in the race to adapt to new environments. On page 441 of this issue, Rowe *et al.* show that evolutionary change in a voltage-gated sodium channel (Nav) drives resistance to painful neurotoxins that enables grasshopper mice (*Onychomys torridus*) in the Arizona desert to prey on an otherwise inaccessible food source, namely bark scorpions (*Centruroides sculpturatus*) (1).

Nociceptors detect a wide range of harmful stimuli, such as mechanical insult, chemicals, acid, or extreme temperature. Yet the nociceptive systems that detect such hazards are remarkably conserved throughout the animal kingdom (2). Nociceptors relay damage signals in the form of action potential trains that travel long distances from skin to spinal cord. Mammalian nociceptors are equipped with a mix of voltage-gated sodium channels that sustain action potential firing. Two critical members of this mix are the tetrodotoxin (TTX)–resistant Nav1.8 and TTX-sensitive Nav1.7 channels (TTX is a pufferfish toxin

Department of Neuroscience, Max Delbrück Center for Molecular Medicine, Robert-Rössle str. 10, 13122 Berlin, Germany. E-mail: glewin@mdc-berlin.de

Grasshopper mice can feed on toxic bark scorpions because they have evolved resistance to the pain normally caused by the toxin in the scorpion sting.



Pain and gain. The bark scorpion toxin activates Nav1.7 channels in grasshopper mice and lab mice. But in grasshopper mice, the toxin potently inhibits the Nav1.8 channel in the same sensory neurons, tipping the balance to inhibit action potential firing in nociceptors. This evolutionary adaptation allows grasshopper mice to feed on bark scorpions with impunity. In contrast, the lab mouse Nav1.8 channel is not blocked by the toxin and the scorpion sting excites nociceptors, causing intense pain.

that potently blocks sodium channels). Both channels are selectively expressed in nociceptors, and gene knockout studies in mice or their mutation in humans are associated with reduced pain or sometimes a complete absence of pain (3).

Rowe *et al.* began by observing that the venom of the bark scorpion, native to the Arizona desert, causes intense behavior indicative of pain in lab mice, probably by activating the Nav1.7 channel. However, grasshopper mice living in the Arizona desert regularly

kill and consume bark scorpions, without any apparent discomfort from multiple stings experienced during prey capture. Recordings from freshly isolated sensory neurons from grasshopper mice and lab mice revealed that the isolated bark scorpion venom strongly activates lab mouse sensory neurons, but strongly inhibited action potential firing in grasshopper mouse sensory neurons.

The authors used pharmacological isolation of the TTX-resistant Nav1.8 current in isolated sensory neurons to show that the

CREDIT: P. HUEY/SCIENCE

scorpion toxin potently inhibited the TTX-resistant Nav1.8 channel current in grasshopper mice neurons, but had no effect on this current in lab mouse sensory neurons (see the figure). They then cloned the Nav1.8 ion channel of the grasshopper mouse to identify the amino acids responsible for the channel block produced by the scorpion venom. A series of elegant domain switching and site-directed mutagenesis studies identified variations in critical residues in domain II, a region adjacent to the pore, that convert Nav1.8 into a venom-sensitive channel. Just switching the sequence order of an acidic glutamic acid (E) and a hydrophilic glutamine (Q) separated by two conserved residues was sufficient to render grasshopper mouse Nav1.8 insensitive to the venom, just like the lab mouse channel.

Animals in the order Rodentia make up more than 40% of all mammalian species and inhabit a vast number of often extreme habitats worldwide. The role of acidic residues in the grasshopper mouse Nav1.8 channel is reminiscent of the way in which another rodent species has coopted charged residues in the Nav1.7 channel to increase inhibition by acid. The subterranean naked mole rat (*Heterocephalus glaber*), indigenous to the dry high plateaus of east Africa, is in no danger from scorpions, but is confronted

with high ambient CO₂ in its crowded underground habitat. High CO₂ is painful, probably because it is converted to acid in exposed tissues. However, acid does not seem to bother naked mole rats, because its Nav1.7 is potently inhibited by acid to block nociceptor firing. Evolution has driven changes in the naked mole rat Nav1.7 channel sequence so that two of three charged residues are altered near the pore to potentially increase channel inhibition by protons (4).

It is striking that both the Nav1.7 and 1.8 channels, which are critical for the propagation of pain information along nociceptor axons to the central nervous system, have undergone strong selective changes in different animals. These changes probably promoted by the extremity of the niches to which the animals have adapted. Scorpion toxins activate nociceptors and produce pain by dramatically slowing the inactivation of sodium channels like Nav1.7. It remains to be shown whether the grasshopper mouse Nav1.7 channel is just as potently activated by components of bark scorpion venom as the same channel is in lab mice. Other factors might contribute to the grasshopper mouse's insensitivity to scorpion venom; for example, Rowe *et al.* found that larger depolarizing impulses were needed to evoke action potentials in sensory

neurons from grasshopper mice relative to those from lab mice.

Pharmaceutical companies have a great interest in developing new analgesic drugs that do what the bark scorpion toxin does in grasshopper mice: prevent pain. Evolution has over millions of years achieved an analgesic strategy tailored to one rodent species. The challenge will be to identify channel sequence variants that have allowed different species to adapt to their unique environments. Gene technology offers the possibility to reverse engineer such variants into lab rodents. Drug designers could well be able to take advantage of the millions of years of natural selection to find new approaches to tackle important drug targets like sodium channels.

References and Notes

1. A. H. Rowe, Y. Xiao, M. P. Rowe, T. R. Cummins, H. H. Zakon, *Science* **342**, 441 (2013).
2. E. S. J. Smith, G. R. Lewin, *J. Comp. Physiol. A* **195**, 1089 (2009).
3. A. Momin, J. N. Wood, *Curr. Opin. Neurobiol.* **18**, 383 (2008).
4. E. S. J. Smith *et al.*, *Science* **334**, 1557 (2011).

Acknowledgments: I am grateful for many helpful comments from members of the Lewin laboratory. The author's work was supported by grants from the European Research Council and Deutsche Forschungsgemeinschaft.

10.1126/science.1244375

CHEMISTRY

Expanding the Scope of Fluorine Tags for PET Imaging

Lin Zhu,^{1,2,3} Karl Ploessl,² Hank F. Kung^{2,4}

Millions of patients undergo PET (positron emission tomography) imaging every year, but there is a critical unmet need for new PET imaging probes capable of diagnosing and monitoring a multitude of diseases. One of the most versatile radionuclides for PET imaging is ¹⁸F, but rapid chemical synthesis of any probe based on ¹⁸F is essential because its physical half-life is only 110 min. Huiban *et al.* (1) recently reported an improved

method for labeling trifluoromethylarenes (Ar-CF₃) with ¹⁸F that could greatly facilitate the development and adoption of new PET probes and allow the site of action of existing drugs to be identified.

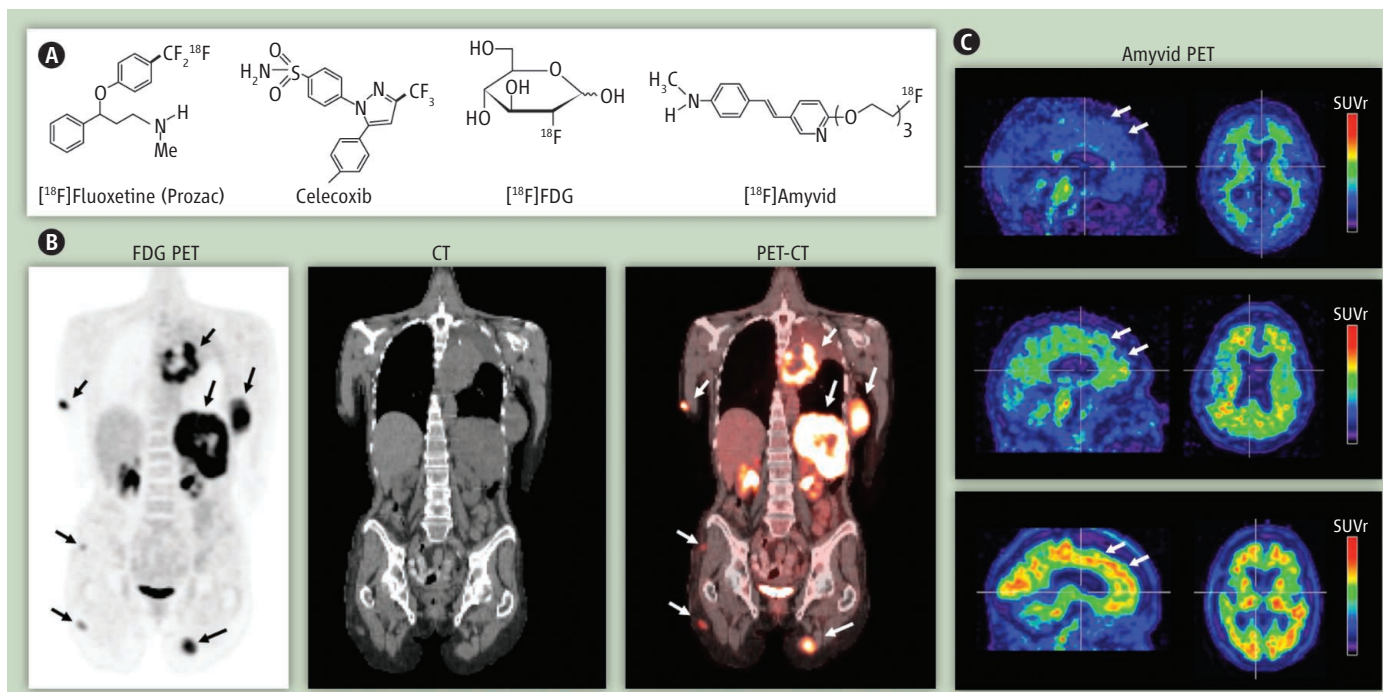
The only PET probe that has achieved widespread clinical application in the United States thus far is 2-[¹⁸F]fluoro-2-deoxy-D-glucose ([¹⁸F]FDG; see the figure, panels A and B), which images cancer metabolism by pinpointing areas of high glucose usage (2, 3). Because cancer cells generally burn extra glucose as fuel, FDG-PET provides clinicians with a diagram of cancer cell activity within the body. This method is now the standard of care in oncology for diagnosis, staging, and monitoring therapy. With the recent development of scanners that combine PET with computed tomography (CT), clinicians

A fast fluorination method can allow a wider range of drug molecules to be imaged in the body with positron emission tomography (PET).

can now compare three-dimensional anatomical CT images with biochemical maps of fuel consumption in cancer tissue. For the past two decades, FDG has been the only ¹⁸F drug approved by the U.S. Food and Drug Administration (FDA) for PET imaging.

Because PET-CT scans can reveal vital information about the anatomical structure within the body and its regional glucose consumption during the same examination, this method has the potential for understanding many other pathophysiological processes. In 2012, the FDA approved a second PET drug, Amyvid (florbetapir f18) (see the figure, panels A and C) (4), for detecting β -amyloid plaques, clumps of proteins that form around neurons in the brain and contribute to the development of Alzheimer's disease (AD). Amyvid is now being used for patient selec-

¹Key Laboratory of Radiopharmaceuticals (Beijing Normal University), Ministry of Education, Beijing 100875, People's Republic of China. ²Department of Radiology, University of Pennsylvania, Philadelphia, PA 19014, USA. ³Beijing Institute for Brain Disorders, Capital Medical University, Beijing, People's Republic of China. ⁴Department of Pharmacology, University of Pennsylvania, Philadelphia, PA 19014, USA. E-mail: kunghf@mail.med.upenn.edu



Tag, inject, image. (A) Chemical structures of fluoxetine, celecoxib, FDG, and Amyvid. (B) The FDG PET-CT fusion images of a cancer patient (melanoma) can provide both anatomical and functional information, thereby pinpointing the cancerous tissue, which shows higher glucose metabolism (arrows). (C) Amyvid-PET images are shown for three subjects, where red is highest standard-

ized uptake value ratio (SUVr): (top row) a normal subject with no β -amyloid plaques; (middle row) moderate load of β -amyloid plaques associated with an early stage of Alzheimer's disease; and (bottom row) high load of β -amyloid plaques (white arrows) indicative of a late stage of Alzheimer's disease (4). [Reproduced from (4)]

tion before enrollment in drug trials specifically designed to reduce the accumulation of β -amyloid plaques in the brain.

Fluorine chemistry and the creation of new ^{18}F tracers now occupy a central role in advancing PET-CT. The fluorine atom is the most electronically negative element; water molecules, through hydrogen bonding, tightly wrap around it. To make the ^{18}F fluoride active for tagging, chemists use a positively charged potassium ion (K^+) caged in a crown ether, to fish out the fluoride ion from water into an organic solvent. The ^{18}F fluoride conjugated with a K^+ -crown ether (a "naked" fluoride) is available for the sort of nucleophilic tagging reaction used to generate ^{18}F FDG and ^{18}F Amyvid.

Because of the short half-life of ^{18}F , ^{18}F FDG is made, distributed, and injected intravenously at a site near the PET-CT. In major medical centers, it is produced by an in-house radiopharmacy; smaller hospitals and clinics obtain FDG from regional radiopharmacies. Commercial vendors have reduced the labeling time to less than 60 min while complying with the FDA's regulations for good manufacturing practice (GMP).

The improved method that Huiban *et al.* reported for tagging Ar-CF_3 with ^{18}F can meet the GMP stringent requirements. They start with the same platform currently used

to make FDG—the naked ^{18}F anion—and in an elegant "one-pot" reaction, generate the intermediate, $^{18}\text{F}\text{CuCF}_3$ via a copper-catalyzed reaction (5) and react this species with aryl iodides to form $\text{Ar-CF}_2\text{-}^{18}\text{F}$. This reaction provides high yields and proceeds in minutes. Their method supplants the nucleophilic fluorination reaction with a ^{18}F fluoride anion currently used for most ^{18}F -labeled tracers, including FDG and Amyvid.

Substitution of a trifluoromethyl group on aromatic rings is a favored tool of medicinal chemists (6–8). The trifluoromethyl group is neutral and compact in size, and because it is stable in the human body, many small molecule-based drugs contain an Ar-CF_3 group, which gives them a longer half-life in the blood and improves efficacy. Two examples of Ar-CF_3 drugs are fluoxetine (Prozac), an antidepressant (selective serotonin reuptake inhibitor, SSRI), and celecoxib (Celebrex), a nonsteroidal anti-inflammatory painkiller (see the figure, panel A). As Huiban *et al.* demonstrated, the Ar-CF_3 group on fluoxetine can be efficiently tagged with their method, and then used with PET imaging to determine where the drug binds and if it is competing for the same binding sites in the brain as other SSRIs.

This capability would be quite useful in future drug development as it would allow

researchers to pinpoint where drugs are distributed within the body to better understand typical drug activity. It would also enable clinicians to personalize therapy and analyze individual responses to treatment. The method developed by Huiban *et al.* promises to facilitate the creation of new probes for PET-CT that will not only enhance the diagnosis of various human diseases but also, in the case of drugs containing an Ar-CF_3 group, help select which patients may be most effectively treated by therapeutic drugs.

References and Notes

1. M. Huiban *et al.*, *Nat. Chem.* **5**, 10.1038/nchem.1756 (2013).
2. C. Plathow, W. A. Weber, *J. Nucl. Med.* **49** (suppl. 2), 435 (2008).
3. R. J. Gillies, I. Robey, R. A. Gatenby, *J. Nucl. Med.* **49** (suppl. 2), 245 (2008).
4. C. M. Clark *et al.*, *JAMA* **305**, 275 (2011).
5. J. G. MacNeil Jr., D. J. Burton, *J. Fluor. Chem.* **55**, 225 (1991).
6. T. Furuya, A. S. Kamlet, T. Ritter, *Nature* **473**, 470 (2011).
7. O. A. Tomashenko, V. V. Grushin, *Chem. Rev.* **111**, 4475 (2011).
8. E. J. Cho *et al.*, *Science* **328**, 1679 (2010).

Acknowledgments: We thank D. Mankoff for supplying the FDG PET-CT images and C. Huang for editorial assistance. Supported in part by grants from Stand-Up 2 Cancer (SU2C), PA Health Department, and NIH (CA-164490).

10.1126/science.1245011

GEOPHYSICS

The New Core Paradox

Peter Olson

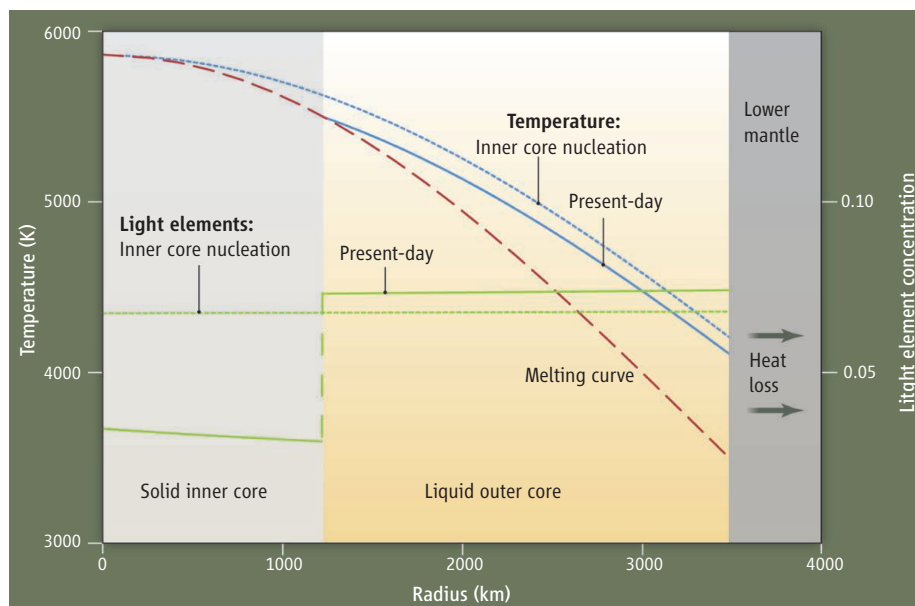
Among the terrestrial planets, Earth is by far the best endowed with energy to drive its internal dynamics. In addition to being the only planet known to have a global system of plate tectonics operating in its crust and mantle, Earth generates a strong magnetic field of its own through self-sustained dynamo action in its iron-rich core. The conventional view is that convection in the liquid outer core provides most of the energy for the geodynamo, and does so not just today, but also in the past, stretching backward in time for at least 3.4 billion years (*1*). Now that view is being challenged by fresh laboratory measurements and first-principles electronic structure calculations, indicating a far greater capacity for the core to transport heat by conduction and, by implication, less ability to transport heat by convection.

The newly calculated values of the thermal conductivity of Earth's core, ranging from 90 to 150 watts per meter per kelvin (W/m/K) (*2, 3*), are appreciably larger than previous, widely used estimates (*4*). Preliminary high-pressure measurements of electrical conductivity, in conjunction with the Wiedemann-Franz law, yield comparable values for thermal conductivity of pure iron, and somewhat lower values of 60 to 100 W/m/K for some of the most likely iron alloys that comprise the outer core (*5–7*).

The seismic structure of the outer core indicates that it is reasonably well mixed, implying that its temperature profile closely follows an adiabat (see the figure). Based on the new thermal conductivity values, the heat conducted down the core adiabat is in the range of 10 to 15 terawatts (TW), nearly equal to, and quite possibly larger than, the total heat flow from the core, which is estimated to be in the range of 8 to 16 TW (*8, 9*).

This difference in total heat flow budget might seem to be a contradiction. In most convecting systems, the total heat flow exceeds that due to conduction alone. However, according to the standard model of the core depicted in the figure, much of the convection in the outer core is compositional, driven by release of buoyant, lighter elements from solidification of the inner core. This compositional convection serves to mix

Recent results show that Earth's core has a large thermal conductivity, raising questions over how the geodynamo evolved.



Evolution of Earth's core. Profiles of temperature and light element concentration versus radius through the core at the present-day (solid line) and at the nucleation time of the inner core (dotted line), compared to the melting curve (dashed line). The temperature profile lies above the melting curve of the iron-rich compounds in the liquid outer core. The intersection of the temperature profile with the melting curve defines the boundary of the solid inner core. As temperature in the core decreases with time due to loss of heat to the lower mantle, the radius of the inner core increases and light elements (Si, S, and O) become concentrated in the outer core. High thermal conductivity in the core implies that the heat conducted down the temperature gradient may exceed the core heat loss. Buoyancy forces derived from the partitioning of light elements and latent heat release from the growing inner core are what drive convective flow in the outer core. (Note that the temperature and composition axis values in the figure are approximate and meant for illustration.)

the outer core, maintains its adiabatic temperature profile, and closes the core's heat balance, meanwhile providing ample kinetic energy for powering the geodynamo. Models of core-mantle interaction (*10*) show little effect from high conductivities on the present-day mantle and, apart from implying an increased rate of inner core growth, little effect on the present-day core.

So, what is the paradox? Problems arise when we consider the past history of the core, before nucleation of the inner core (estimated to have begun within the past one billion years (*7*)). Without the energy supplied by inner core growth, the geodynamo would have been even more reliant on thermal convection, which the new high thermal conductivity determinations cast doubt on.

The best way around this paradox is to think beyond the standard model of core evolution. The core-mantle boundary is so heterogeneous (*11*) that even if the outer core

is stably stratified on average, thermal convection can occur in localized regions, and dynamo action remains possible under these circumstances (*12*). There are also alternative energy sources that the core might have tapped in its past—for example, instabilities due to precession and tides; other forms of compositional convection involving less soluble light elements; and protracted core formation. And, last but not least, additional high-pressure, high-temperature measurements are needed to pin down the actual conductivity of the core.

For other terrestrial planet dynamos, high thermal conductivity offers a ready (if simplistic) explanation for why so many of these failed in the deep past. For the geodynamo, this is not the first time the viability of the convective mechanism has been questioned. That honor goes back 40 years, to the original "core paradox" (*13*) that had assumed an anomalously low melting temperature gradient in the core. Diamond anvil cell mea-

Earth and Planetary Sciences, Johns Hopkins University, Baltimore, MD 21218, USA. E-mail: olson@jhu.edu

surements on iron subsequently showed this assumption to be wrong (14), demonstrating (yet again) the importance of well-constrained deep Earth parameters.

References

1. J. A. Tarduno *et al.*, *Science* **327**, 1238 (2010).
2. N. de Koker, G. Steinle-Neumann, V. Vlček, *Proc. Natl. Acad. Sci. U.S.A.* **109**, 4070 (2012).
3. M. Pozzo, C. Davies, D. Gubbins, D. Alfè, *Nature* **485**, 355 (2012).
4. F. D. Stacey, D. E. Loper, *Phys. Earth Planet. Inter.* **161**, 13 (2007).
5. L. Deng, C. Seagle, Y. Fei, A. Shahar, *Geophys. Res. Lett.* **40**, 33 (2013).
6. C. T. Seagle *et al.*, *Electrical and Thermal Transport Properties of Iron and Iron-Silicon Alloy at High Pressure* (No. SAND2012-83211), Sandia National Laboratories <http://dx.doi.org/10.1002/grl.51014> (2012).
7. H. Gomi *et al.*, *Phys. Earth Planet. Inter.*, <http://dx.doi.org/10.1016/j.pepi.2013.07.010> (2013).
8. T. Lay, J. Hernlund, B. A. Buffett, *Nat. Geosci.* **1**, 25 (2008).
9. B. Wu, P. Driscoll, P. Olson, *J. Geophys. Res. Solid Earth* **116**, 1978 (2011).
10. T. Nakagawa, P. J. Tackley, *Geophys. Res. Lett.* **40**, 2652 (2013).
11. R. D. van der Hilst *et al.*, *Science* **315**, 1813 (2007).
12. P. Olson, R. Deguen, L. Hinnov, S. Zhong, *Phys. Earth Planet. Inter.* **214**, 87 (2013).
13. G. C. Kennedy, G. H. Higgins, *J. Geophys. Res.* **78**, 900 (1973).
14. S. Anzellini, A. Dewaele, M. Mezour, P. Loubeyre, G. Morard, *Science* **340**, 464 (2013).

10.1126/science.1243477

IMMUNOLOGY

Mucus Coat, a Dress Code for Tolerance

Yasmine Belkaid and John Grainger

The gastrointestinal tract is home to the highest density of commensal bacteria in the human body and is a primary site of pathogen exposure. Understanding how the immune system recognizes and responds to friend or foe in the gut is central to developing treatments for allergic and inflammatory diseases. Both specialized cells and structural compartmentalization of the gut are key controllers of immune responses. In particular, a mucus layer covers the entire gastrointestinal tract, physically separating the microbiota from host tissue and preventing pathogen invasion (1). On page 447 of this issue, Shan *et al.* (2) reveal that mucus does more than act as a shield—it also influences the function of intestinal antigen-presenting cells and epithelial cells to sustain the ability of the host to maintain tolerance toward food and commensal antigens.

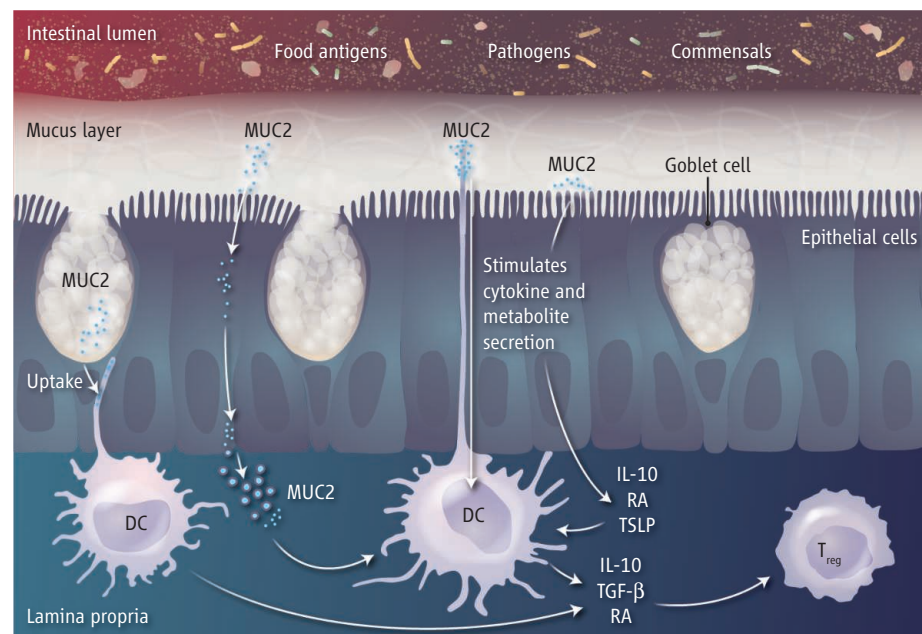
A cardinal feature of tissues is their capacity to respond to defined challenges in a site-specific manner, an especially important attribute for barriers to the external environment, such as the gut or lung. This characteristic is essential for inducing immune responses in a manner that preserves the physiological and functional requirements of a defined microenvironment. As such, subsets of cells seed, and are locally “conditioned” by, tissue-specific factors. A few such molecules have been identified including transforming growth factor- β (TGF- β), the vitamin A metabolite retinoic acid, and thymic stromal lymphopoietin (TSLP).

Program in Barrier Immunity and Repair, Mucosal Immunology Section, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, MD 20892, USA. E-mail: ybelkaid@niaid.nih.gov

Shan *et al.* show that the gut mucus provides a key microenvironmental signal that educates dendritic cells (DCs) to develop tolerance toward food and commensal antigens. DCs lie beneath the intestinal epithelial cell layer and present antigens to other cells of the immune system. Exposure of DCs to mucin-2 (MUC2), a major mucus component, subdues their responses to microbe-derived signals and also promotes their capacity to stimulate the production of

Mucus is a determinant of gut immune specification and immune tolerance.

regulatory T cells (T_{reg}), key determinants of oral tolerance (3, 4). In particular, Shan *et al.* observed that MUC2 increased the expression of factors by DCs that allow them to induce T_{reg} production such as TGF- β and retinaldehyde dehydrogenase, an enzyme involved in generating retinoic acid (5). Moreover, MUC2 also acted directly on epithelial cells to stimulate their release of molecules that support DC regulatory function. The ability of MUC2 to promote regu-



Gut mucus. Mucus segregates commensal microbiota from the intestinal epithelium. Dendritic cells (DCs) may be exposed to MUC2 by direct uptake, through interactions with goblet cells, or through MUC2-containing vesicles released by epithelial cells. Mucus sensing enhances DC tolerogenic functions, including the release of TGF- β , retinoic acid (RA), and interleukin 10 (IL-10), factors that induce T_{reg} cell production. MUC2 can also interact with epithelial cells and support the production of factors [thymic stromal lymphopoietin (TSLP), IL-10, and RA] that further promote the capacity of DCs to induce regulatory responses.

CREDIT: H. McDONALD/SCIENCE

latory responses depends on the interaction of glycan moieties present on MUC2 with galectin-3 (a carbohydrate-binding protein) and the capacity of this complex to engage Dectin-1 on the surface of DCs. Dectin-1, a C-type lectin expressed on the surface of DCs, is a pattern recognition receptor previously shown to be associated with antifungal immune responses. The downstream effect of Dectin-1 interaction with MUC-2 is the expression of β -catenin, a factor that can control the tolerogenic function of gut DCs (6). These results imply that by amplifying various aspects of the gut regulatory network, mucus may be a central determinant of gut immune specification and immune tolerance (see the figure).

Many commensal bacteria use mucus as a source of carbohydrates and express adhesins with specificities for mucus glycans to facilitate this binding (1). One hypothesis, based on the findings of Shan *et al.*, is that in the healthy gut, commensals will be coated in MUC2 and therefore only be seen in the context of a tolerogenic signal.

The mechanism by which DCs are exposed to glycosylated MUC2 in the gut environment remains unclear. Such exposure may result from direct collection of MUC2 through extension of dendrites into the lumen (7). MUC2 sensing may also represent a mechanism by which DCs assess epithelial cell status. In this regard, epithelial cells can recycle mucins and could release mucin-containing vesicles basolaterally (8). This may condition DCs beneath the epithelium to drive the production of T_{reg} specific to food or commensal antigens. Furthermore, DCs can directly take up antigen from the very same cells that produce MUC2 (goblet cells). This could provide another route for direct coupling of tolerogenic signals with antigen sampling (9).

A corollary to the findings of Shan *et al.* is that impaired or aberrant mucus production that is often associated with inflammatory responses may have profound consequences for tissue homeostasis, not only because of enhanced contact with commensals but also as a result of alterations to DC function. Indeed, acute mucosal infections—during which tolerogenic responses to food and commensals are abrogated (10)—are also associated with goblet cell hypoplasia and dramatic reductions in mucus production (11). By contrast, parasitic worm infections that are linked to enhanced mucus generation are also associated with increased regulatory responses and enhanced tolerance to environmental antigens (12).

The findings of Shan *et al.* are of particular relevance to understanding severe inflamma-

tory conditions such as inflammatory bowel diseases. Changes to mucus glycosylation can occur during inflammatory responses, and altered glycosylation of mucus has been associated with colitis (13). Moreover, variants of FUT2, a glycosyltransferase that modifies mucins, are linked to enhanced susceptibility to Crohn's disease (an inflammatory bowel disease) (14). Because of the physical protection afforded by mucus, strategies that restore its integrity in inflammatory diseases are an active area of research. Understanding the signals driven by tissue-specific mucins may open a new approach to designing therapies that restore tissue homeostasis and tolerance at barrier sites, such as the gastrointestinal tract or the lung.

References and Notes

1. G. C. Hansson, *Curr. Opin. Microbiol.* **15**, 57 (2012).
2. M. Shan *et al.*, *Science* **342**, 447 (2013); 10.1126/science.1237910.
3. C. M. Sun *et al.*, *J. Exp. Med.* **204**, 1775 (2007).
4. J. L. Coombes *et al.*, *J. Exp. Med.* **204**, 1757 (2007).
5. M. Iwata *et al.*, *Immunity* **21**, 527 (2004).
6. S. Manicassamy *et al.*, *Science* **329**, 849 (2010).
7. J. Farache *et al.*, *Immunity* **38**, 581 (2013).
8. A. J. Müller *et al.*, *Cell Host Microbe* **11**, 19 (2012).
9. J. R. McDole *et al.*, *Nature* **483**, 345 (2012).
10. T. W. Hand *et al.*, *Science* **337**, 1553 (2012).
11. M. Raetz *et al.*, *Nat. Immunol.* **14**, 136 (2013).
12. M. S. Wilson *et al.*, *J. Exp. Med.* **202**, 1199 (2005).
13. J. M. Larsson *et al.*, *Inflamm. Bowel Dis.* **17**, 2299 (2011).
14. P. Rausch *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **108**, 19030 (2011).

Acknowledgements: This work was supported by the Division of Intramural Research, National Institute of Allergy and Infectious Diseases.

10.1126/science.1246252

ENVIRONMENTAL SCIENCE

Blooms Bite the Hand That Feeds Them

Hans W. Paerl¹ and Timothy G. Otten^{1,2}

A positive feedback exists between eutrophication and the global proliferation of toxin-producing cyanobacterial blooms.

In recent decades, nutrient over-enrichment (eutrophication) and climate-change effects have led to a rise in toxin-producing cyanobacterial harmful algal blooms (CyanoHABs) in freshwater systems worldwide (1). Microcystin, the most ubiquitous cyanotoxin, is a serious drinking water threat due to its potent liver toxicity and carcinogenic potential (2). The distribution of microcystin synthetase (*mcy*) genes across many genera suggests that production of this metabolite was a widely shared trait early in cyanobacterial evolution (3). However, not all cyanobacterial strains have retained this pathway. There is growing evidence that oxidative stressors in high-irradiance surface waters, where blooms accumulate, select for toxigenic strains over their nontoxic counterparts.

In eutrophic systems, toxigenic strains can constitute the majority of bloom populations. This pattern is most pronounced from late spring through summer, whereas fall populations are often dominated by nontoxic strains (4–6). Thus, the toxigenic strains dominate when water residence time and temperature

are greatest, the water column is strongly stratified, solar radiation is highest, and the days are longest. In contrast, under mixed conditions, nontoxic strains are superior competitors due to their different photopigment contents and higher affinity for light absorption (7).

Differences in sensitivities to reactive oxygen species may explain the pattern of toxigenic strain dominance during periods of high light intensity and lake stratification. Reactive oxygen species are produced by ultraviolet (UV) radiation, which photocatalyzes dissolved organic carbon in surface waters into superoxide (O_2^-) and hydrogen peroxide (H_2O_2) (8). The amount of H_2O_2 generated is proportional to the dissolved organic carbon concentration and light intensity; hence, reactive oxygen species more readily accumulate in calm surface waters than at depth or during periods of mixing. Like molecular oxygen, H_2O_2 passively diffuses into cells and is an important external source of oxidative stress.

In addition to externally generated oxidative stressors, all aerobic organisms produce reactive oxygen species as metabolic by-products. Algae and higher plants generate additional oxidative stressors through pho-

¹Institute of Marine Sciences, University of North Carolina at Chapel Hill, Morehead City, NC 28557, USA. ²Department of Microbiology, Oregon State University, Corvallis, OR 97331, USA. E-mail: hans_paerl@unc.edu

latory responses depends on the interaction of glycan moieties present on MUC2 with galectin-3 (a carbohydrate-binding protein) and the capacity of this complex to engage Dectin-1 on the surface of DCs. Dectin-1, a C-type lectin expressed on the surface of DCs, is a pattern recognition receptor previously shown to be associated with antifungal immune responses. The downstream effect of Dectin-1 interaction with MUC-2 is the expression of β -catenin, a factor that can control the tolerogenic function of gut DCs (6). These results imply that by amplifying various aspects of the gut regulatory network, mucus may be a central determinant of gut immune specification and immune tolerance (see the figure).

Many commensal bacteria use mucus as a source of carbohydrates and express adhesins with specificities for mucus glycans to facilitate this binding (1). One hypothesis, based on the findings of Shan *et al.*, is that in the healthy gut, commensals will be coated in MUC2 and therefore only be seen in the context of a tolerogenic signal.

The mechanism by which DCs are exposed to glycosylated MUC2 in the gut environment remains unclear. Such exposure may result from direct collection of MUC2 through extension of dendrites into the lumen (7). MUC2 sensing may also represent a mechanism by which DCs assess epithelial cell status. In this regard, epithelial cells can recycle mucins and could release mucin-containing vesicles basolaterally (8). This may condition DCs beneath the epithelium to drive the production of T_{regs} specific to food or commensal antigens. Furthermore, DCs can directly take up antigen from the very same cells that produce MUC2 (goblet cells). This could provide another route for direct coupling of tolerogenic signals with antigen sampling (9).

A corollary to the findings of Shan *et al.* is that impaired or aberrant mucus production that is often associated with inflammatory responses may have profound consequences for tissue homeostasis, not only because of enhanced contact with commensals but also as a result of alterations to DC function. Indeed, acute mucosal infections—during which tolerogenic responses to food and commensals are abrogated (10)—are also associated with goblet cell hypoplasia and dramatic reductions in mucus production (11). By contrast, parasitic worm infections that are linked to enhanced mucus generation are also associated with increased regulatory responses and enhanced tolerance to environmental antigens (12).

The findings of Shan *et al.* are of particular relevance to understanding severe inflamma-

tory conditions such as inflammatory bowel diseases. Changes to mucus glycosylation can occur during inflammatory responses, and altered glycosylation of mucus has been associated with colitis (13). Moreover, variants of FUT2, a glycosyltransferase that modifies mucins, are linked to enhanced susceptibility to Crohn's disease (an inflammatory bowel disease) (14). Because of the physical protection afforded by mucus, strategies that restore its integrity in inflammatory diseases are an active area of research. Understanding the signals driven by tissue-specific mucins may open a new approach to designing therapies that restore tissue homeostasis and tolerance at barrier sites, such as the gastrointestinal tract or the lung.

References and Notes

1. G. C. Hansson, *Curr. Opin. Microbiol.* **15**, 57 (2012).
2. M. Shan *et al.*, *Science* **342**, 447 (2013); 10.1126/science.1237910.
3. C. M. Sun *et al.*, *J. Exp. Med.* **204**, 1775 (2007).
4. J. L. Coombes *et al.*, *J. Exp. Med.* **204**, 1757 (2007).
5. M. Iwata *et al.*, *Immunity* **21**, 527 (2004).
6. S. Manicassamy *et al.*, *Science* **329**, 849 (2010).
7. J. Farache *et al.*, *Immunity* **38**, 581 (2013).
8. A. J. Müller *et al.*, *Cell Host Microbe* **11**, 19 (2012).
9. J. R. McDole *et al.*, *Nature* **483**, 345 (2012).
10. T. W. Hand *et al.*, *Science* **337**, 1553 (2012).
11. M. Raetz *et al.*, *Nat. Immunol.* **14**, 136 (2013).
12. M. S. Wilson *et al.*, *J. Exp. Med.* **202**, 1199 (2005).
13. J. M. Larsson *et al.*, *Inflamm. Bowel Dis.* **17**, 2299 (2011).
14. P. Rausch *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **108**, 19030 (2011).

Acknowledgements: This work was supported by the Division of Intramural Research, National Institute of Allergy and Infectious Diseases.

10.1126/science.1246252

ENVIRONMENTAL SCIENCE

Blooms Bite the Hand That Feeds Them

Hans W. Paerl¹ and Timothy G. Otten^{1,2}

A positive feedback exists between eutrophication and the global proliferation of toxin-producing cyanobacterial blooms.

In recent decades, nutrient over-enrichment (eutrophication) and climate-change effects have led to a rise in toxin-producing cyanobacterial harmful algal blooms (CyanoHABs) in freshwater systems worldwide (1). Microcystin, the most ubiquitous cyanotoxin, is a serious drinking water threat due to its potent liver toxicity and carcinogenic potential (2). The distribution of microcystin synthetase (*mcy*) genes across many genera suggests that production of this metabolite was a widely shared trait early in cyanobacterial evolution (3). However, not all cyanobacterial strains have retained this pathway. There is growing evidence that oxidative stressors in high-irradiance surface waters, where blooms accumulate, select for toxigenic strains over their nontoxic counterparts.

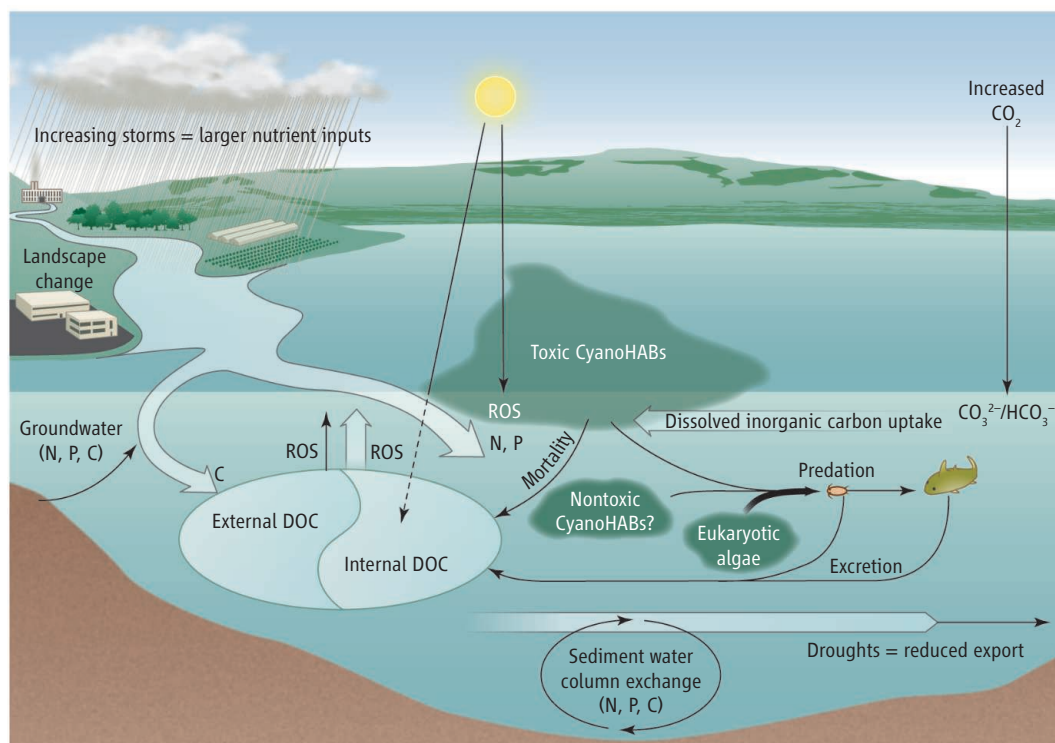
In eutrophic systems, toxigenic strains can constitute the majority of bloom populations. This pattern is most pronounced from late spring through summer, whereas fall populations are often dominated by nontoxic strains (4–6). Thus, the toxigenic strains dominate when water residence time and temperature

are greatest, the water column is strongly stratified, solar radiation is highest, and the days are longest. In contrast, under mixed conditions, nontoxic strains are superior competitors due to their different photopigment contents and higher affinity for light absorption (7).

Differences in sensitivities to reactive oxygen species may explain the pattern of toxigenic strain dominance during periods of high light intensity and lake stratification. Reactive oxygen species are produced by ultraviolet (UV) radiation, which photocatalyzes dissolved organic carbon in surface waters into superoxide (O_2^-) and hydrogen peroxide (H_2O_2) (8). The amount of H_2O_2 generated is proportional to the dissolved organic carbon concentration and light intensity; hence, reactive oxygen species more readily accumulate in calm surface waters than at depth or during periods of mixing. Like molecular oxygen, H_2O_2 passively diffuses into cells and is an important external source of oxidative stress.

In addition to externally generated oxidative stressors, all aerobic organisms produce reactive oxygen species as metabolic by-products. Algae and higher plants generate additional oxidative stressors through pho-

¹Institute of Marine Sciences, University of North Carolina at Chapel Hill, Morehead City, NC 28557, USA. ²Department of Microbiology, Oregon State University, Corvallis, OR 97331, USA. E-mail: hans_paerl@unc.edu



Toxic feedback. Cultural eutrophication and climate change promote cyanobacterial harmful algal blooms (CyanoHABs). Blooms increase reactive oxygen species (ROS) directly via photosynthesis, and indirectly by augmenting the dissolved organic carbon (DOC) pool in lake surface waters, which is in turn UV photocatalyzed into ROS. This feedback selects for toxin-producing cyanobacterial strains.

and longer water residence times due to droughts—exacerbate a positive-feedback loop that competitively displaces nontoxic cyanobacterial strains (see the figure). As cyanobacterial-derived dissolved organic carbon inputs into lakes rise, the concentrations of UV-photocatalyzed oxidative stressors are expected to also increase, intensifying the selective pressure that favors microcystin producers.

Future studies are needed

tosynthesis that may “leak” out of the cell. Intracellular build-up of reactive oxygen species leads to oxidative damage of DNA, proteins, and lipids; therefore, redox equilibrium is paramount for cell fitness.

How do buoyant cyanobacteria thrive in the part of the water column where reactive oxygen species are most abundant? All cyanobacteria rely on multiple antioxidant pathways, but toxigenic strains have an additional advantage. There is now compelling evidence that microcystin has a protective role in the oxidative stress response of cyanobacteria. *In vivo*, microcystins covalently bind to proteins, including components involved in photosynthesis and carbon sequestration, during oxidative stress; these bound protein complexes resist proteolytic degradation (9). Furthermore, a *mcy* gene knockout strain was more adversely affected by high light intensities and H_2O_2 additions than the microcystin-producing parent strain (9). In an ecologic context, these findings suggest that under oxidative stress conditions, toxin-producing strains enjoy a competitive growth advantage over nontoxic ones. Experimental evidence corroborates this supposition. In mixed-culture competition experiments, warmer temperatures and oxidative stress favor dominance of toxic genotypes (7).

Dissolved organic carbon concentrations are increasing in lakes around the globe (10), causing reactive oxygen concentrations to rise in surface waters. A lake's total dis-

solved organic carbon is determined by external (allochthonous) input, internal (autochthonous) generation by carbon fixation, sedimentation, mineralization, and export (11). Allochthonous dissolved organic carbon has long been assumed to be the major contributor to the lake carbon budget (12). However, in highly productive systems such as those impacted by CyanoHABs, autochthonous carbon is more important to the aquatic microbiome for several reasons (13).

First, allochthonous inputs of dissolved organic carbon are resistant to change, because most will have undergone bacterial or UV transformation prior to lake deposition. Second, CyanoHABs are autotrophs, which assimilate inorganic carbon (CO_2) into organic carbon and therefore represent a large potential pool of labile DOC upon cell death. Third, CyanoHABs are ineffectively grazed by zooplankton; their major population losses therefore come from viral and bacterial lysis and programmed cell death. In culture-based experiments, as little as 3% cell mortality can generate more than 50% of the total dissolved organic carbon content, and natural mortality is estimated at ~0.5% per day (14). Finally, long water residence times, which favor bloom formation, also signify low export rates of internally derived dissolved organic carbon following cell mortality events.

It follows from these considerations that aspects of climate change—warmer temperatures resulting in stronger stratification,

to resolve the fate of dissolved organic carbon derived from CyanoHABs and to determine whether toxigenic and nontoxic strains exhibit unique niche adaptation strategies. Any increase in the proportion of toxigenic cells in a bloom event has important public health implications. Fortunately, several decades of limnological investigations have yielded management options for mitigating CyanoHABs (15) that should reduce the self-amplifying impacts of this positive-feedback loop.

References

1. H. W. Paerl, J. Huisman, *Science* **320**, 57 (2008).
2. W. W. Carmichael, *J. Appl. Microbiol.* **72**, 445 (1992).
3. A. Rantala et al., *Proc. Natl. Acad. Sci. U.S.A.* **101**, 568 (2004).
4. T. W. Davis, D. L. Berry, G. L. Boyer, C. L. Gobler, *Harmful Algae* **8**, 715 (2009).
5. T. G. Otten, H. Xu, B. Qin, G. Zhu, H. W. Paerl, *Environ. Sci. Technol.* **46**, 3480 (2012).
6. C. S. Bozarth, A. D. Schwartz, J. W. Shepardson, F. S. Colwell, T. W. Dreher, *Appl. Environ. Microbiol.* **76**, 5207 (2010).
7. W. E. A. Kardinaal et al., *Appl. Environ. Microbiol.* **73**, 2939 (2007).
8. W. J. Cooper, R. G. Zika, *Science* **220**, 711 (1983).
9. Y. Zilliges et al., *PLOS ONE* **6**, e17615 (2011).
10. C. D. Evans, D. T. Monteith, D. M. Cooper, *Environ. Pollut.* **137**, 55 (2005).
11. P. C. Hanson et al., *PLOS ONE* **6**, e21884 (2011).
12. D. L. Bade et al., *Biogeochemistry* **84**, 115 (2007).
13. M. C. Horner-Devine, M. A. Leibold, V. H. Smith, B. J. M. Bohannan, *Ecol. Lett.* **6**, 613 (2003).
14. D. Y. Lee, G. Y. Rhee, *J. Phycol.* **33**, 991 (1997).
15. H. W. Paerl, N. S. Hall, E. S. Calandrino, *Sci. Total Environ.* **409**, 1739 (2011).

10.1126/science.1245276

CREDIT: P. HUEY/SCIENCE; ADAPTED FROM A. JOYNER/UNIVERSITY OF NORTH CAROLINA

Radiation and Atomic Literacy for Nonscientists

Andy Johnson

Inquiry into Radioactivity, an IBI prize-winning module, enables students to develop meaningful understandings of ionizing radiation through guided experimentation.

The student teams stand outside of their closed classroom door holding Geiger counters. Behind the door, the classroom has been salted with radioactive antiques, rocks, and commercially available sources. Their mission: safely locate the radioactive objects and quantify their radiation levels. Keenly aware that, thus far, they have only encountered background radiation, the team members wait expectantly, some uneasily, for this new task. Some of the team members are smiling, anticipating an experience something like a postapocalyptic Easter egg hunt. What will they find behind the door?

A scene such as this is just another day in a classroom using the Inquiry into Radioactivity (IiR) course materials. The topics of radioactivity and ionizing radiation tend to be taught briefly, if at all, in science courses for nonmajors. However, ionizing radiation is becoming increasingly important in modern life. Gamma scintigraphy and x-ray computed tomography scans are increasingly used in medicine, industrial uses of radiation are proliferating, and the meltdown of the nuclear reactor at Fukushima has raised the visibility of radioactive hazards.

A society that uses nuclear technology needs to be radiation-literate. The IiR Project of the Center for Math and Science Education at Black Hills State University is developing tools and techniques to raise the level of radiation literacy among college and high school students who typically avoid science.

Most people do not know what radiation is. We, like other researchers, have found that students and the general public do not correctly understand ionizing radiation as



Students discuss whether apples on a radioactive antique plate will become radioactive.

high-speed particles emitted from radioactive atomic nuclei (1). Instead, most view radioactivity as its own sort of matter and typically do not distinguish between radiation and radioactive material. They think of both as something that spreads out from a source and affects other objects in the vicinity (2). Radiation is seen as “bad stuff,” like dirt or germs, that “contaminates things.” This general set of ideas prevails in the United States and Europe among all levels of introductory physics students (3), which makes it unlikely that students can reason meaningfully about radiation.

However, students can understand radioactivity without an extensive background in science. In order to make sense of ionizing radiation, students need to know what ionizing radiation is, where it comes from, and how it can do harm. Those three categories have guided the development of the IiR materials. And, although a number of IiR activities, including the radioactive object hunt, are fun for students, the goal of developing a functional understanding of ionizing radiation drives everything that happens in the classroom.

The IiR Project began in 2004 with the question “How much can students figure out about radiation by doing inquiry?” After years of development and classroom testing,

our answer is, “quite a bit!” The teacher just has to present the students with the right experiences, questions, and classroom structure. The guided inquiry approach is used because students develop deeper understanding of science through interactive engagement methods such as inquiry (4, 5). Moreover, it encourages students to learn more than scientific content—students develop scientific reasoning abilities as a natural consequence of learning science (6), and, a selfish motivation, the interaction of students in the classroom is much more engaging and enjoyable for everyone involved, including the teacher (7).

By exploring the classroom with cheap electromagnetic field detectors, each made from a guitar amplifier and a coil, students in the IiR classroom discover that electrical devices emit a different type of radiation that is not emitted from radioactive sources. By experimenting with an infrared remote control, a light sensor, and microphones, students determine that there are many different types of radiation, but only one type is detected by Geiger counters. Students test contamination by ionizing radiation directly by taping test objects—such as coins, pencils, or food—to classroom radioactive sources and checking their radiation counts after a weekend has passed (see the first figure). Many are very surprised (and a little disappointed!) that their test objects never become radioactive. By checking the penetration of radiation through different objects, students discover three types of ionizing radiation—alpha, beta, and gamma—and they are surprised to find that count rate and penetrating power are not connected.

Part of the challenge in developing a particle view of radioactivity is the need to understand that atoms emit radiation particles but are also damaged by radiation via ionization. We found that our students initially understand very little about atoms, despite previous education on the topic (8). Thus, the IiR materials explicitly focus on the structure and

Center for Advancement of Math and Science Education (CAMSE), Black Hills State University, 1200 University Street, Spearfish, SD 57799, USA. E-mail: andy.johnson@bhsu.edu

*IBI, Science Prize for Inquiry-Based Instruction; www.sciencemag.org/site/feature/data/prizes/inquiry/.

properties of atoms and on the interaction of ionizing radiation with matter. This required the development of new tools that enable students to do experiments on single atoms. The project designed special pedagogical simulators that reveal the invisible behavior of atoms. These simulators give results the same way macroscopic experiments would—phenomena are displayed in detail but no explanations are provided. Strategically designed questions in the course materials support students developing scientific ideas about atoms.

We developed a special Atom Builder simulation that allows students to “build” every known isotope from hydrogen to dubnium (element 105) (see the second figure). Students build atoms and then send them to a test world in which ions attract and repel each other, and unstable isotopes explode, releasing various particles. Students are drawn to Atom Builder by its gamelike look and feel. They want to play with it, and in doing so, they learn about atoms. Using the Atom Builder simulator in conjunction with the LiR materials, students develop useful, meaningful understandings of atoms, ions, and nuclear stability (8). Atom Builder and the other LiR simulators are available at www.camse.org/sims.

Later, when studying the interaction of radiation with matter, students use two other

About the author



Andy Johnson is associate director for Science Education at the South Dakota Center for Math and Science Education at Black Hills State University and a physics education researcher trained in inquiry physics teaching and classroom research techniques. In addition to creating inquiry-based course materials, he teaches physics to college students and provides professional development to kindergarten through high-school teachers. Johnson received a B.S. in Physics at Colorado School of Mines, an M.S. in Physics at Arizona State University, and a Ph.D. in Physics Education at San Diego State University and the University of California at San Diego. Some of his students gripe about finishing each class exhausted from thinking hard, but many have also declared that the first time they ever understood science was in Johnson’s class.

LiR simulators: (i) Atom Invaders, which allows students to shoot alphas, betas, gammas, and neutrons at individual atoms and molecules, and (ii) Tracks, which simulates the interaction of alpha, beta, and gamma radiation with everyday objects at three different size scales. Students use these to work out how radiation interacts with matter. The class gradually invents an explanation that radiation particles cause damage at the molecular scale by removing electrons from many molecules, often breaking molecular bonds. Developing this mechanism for the fundamental process of radiation damage enables the unit to continue with lessons on how radiation can damage DNA and cells. Additional LiR activities provide information on acute and stochastic radiation doses and medical uses of radiation.

Using LiR we find that the majority of students do not transition to the moving-particle view of radiation until they investigate emission and ionization at the atomic scale (9). By studying the ways that students spoke and wrote about radiation and the effects of exposure throughout a semester, we discovered that many students only abandoned their ideas of “radiation as stuff” and “contamination by radiation” after they completed investigations that revealed that the particle view can explain both the origin of radiation in nuclei and ionization effects.

Our findings suggest that a brief study of radiation and radioactivity (over a few weeks) will not lead students to shift their thinking about what radiation is. Regardless of the clarity or quality of the presentation, students will likely persist in thinking of radiation as having material-like properties

that cause contamination. To come to understand what radiation is, most students apparently have to think long and hard about where radiation comes from and how it does harm. The LiR materials accomplish this by involving students in actively developing mechanistic models of radiation, atoms, radioactive decay, and ionization. Although this requires a substantial amount of class time, the effort results in radiation-literate students who carry with them deeper understandings of the world at the atomic scale.

References and Notes

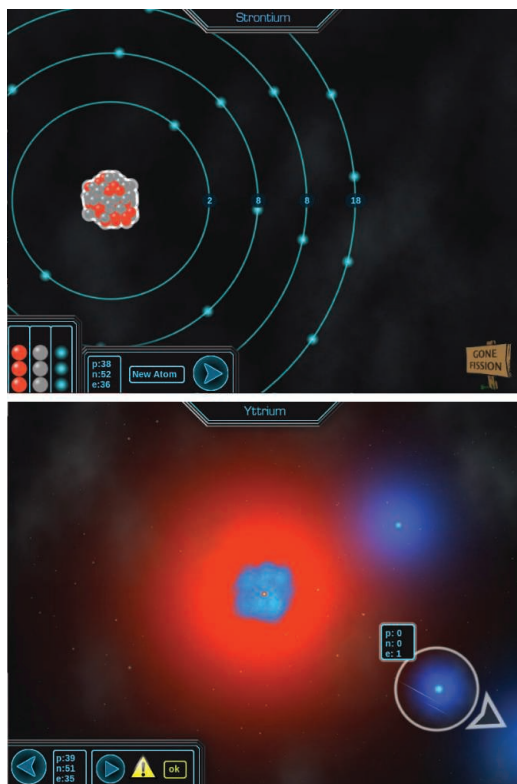
1. H. M. C. Eijkelhof, Radiation and Risk in Physics Education (University of Utrecht, Utrecht, 1990); www.iaea.org/inis/collection/NCLCollectionStore/_Public/22/010/22010294.pdf.
2. R. Millar, J. S. Gill, *Phys. Educ.* **31**, 27 (1996).
3. E. Prather, R. Harrington, *J. Coll. Sci. Teach.* **31**, 89 (2001).
4. R. Hake, *Am. J. Phys.* **66**, 64 (1998).
5. K. Malone, *Phys. Rev. Special Top. Phys. Ed. Res.* **4**, 020107 (2008).
6. E. Etkina et al., *J. Learn. Sci.* **19**, 54 (2010).
7. E. Brewster, L. Kramer, G. O’Brien, *Phys. Rev. Special Top. Phys. Ed. Res.* **5**, 013102 (2009).
8. A. Johnson, A. Hafele, *Proceedings of the 2010 Physics Education Research Conference*, Portland OR, 21 to 22 July 2010 (American Institute of Physics, College Park, MD, 2010).
9. R. Maird et al., *Proceedings of the 2012 National Conference on Undergraduate Research*, Weber State College, Ogden, UT, 29 to 31 March 2012 (Univ. North Carolina, Asheville, 2012); www.camse.org/radiation/pubs/Differentiation2012.pdf.

Acknowledgments: The author thanks his student researchers, especially A. Hafele, for crucially important assistance; his physics students for allowing him to study how they learned about radioactivity; F. Johnson for excellent software user interface work; F. Goldberg for opportunities to learn about teaching physics; and the NSF for funding the LiR project. The Inquiry into Radioactivity Project is supported by NSF DUE grant 0942699. Any opinions, findings, and conclusions or recommendations expressed in this article are those of the author and do not necessarily reflect the views of the NSF.

Supplementary Materials

www.sciencemag.org/content/342/6157/436/suppl/DC1

10.1126/science.1230003



The Atom Builder simulator. In the lower frame, strontium-90 has transformed to yttrium-90, emitted a beta, and self-ionized.

CREDIT: CAROLE JOHNSON



NEUROSCIENCE

Will Brain Stimulation Technology Lead to “Neuroenhancement”?

Restoration of normal function has driven development of devices such as cochlear implants for deafness, deep-brain stimulators for Parkinson’s disease, and bionic eyes for the blind, but there has long been a fascination with using similar technologies to “neuroenhance” healthy individuals, helping them control emotions, improve memory and cognition, and even communicate wordlessly with others.

Speakers at a 19 September session on neuroenhancement co-hosted by AAAS and the Dana Foundation urged separating hype from hope and stressed the value of currently available neurostimulation devices to improve the lives of those with disabling

Both in science and science fiction, the specter of neural enhancement and control has long been debated. Joseph Pancrazio, head of the department of bioengineering at George Mason University, noted the work of Jose Delgado of Yale University in the 1960s. He used an implant to stimulate a region of a bull’s brain called the caudate nucleus. Delgado stepped into a bull ring and, when the bull charged, pressed a remote control button that caused the animal to stop in its tracks.

Delgado claimed that the stimulus caused the bull to lose its aggressive instinct and spoke of a future when such methods would allow “a less cruel, happier, and better man.”

Michael Crichton, a student of one of Delgado’s collaborators, offered an alternative view. In his novel *The Terminal Man*, a man with an electrical implant to control his epilepsy becomes a vicious killer.

While the fascination with neuroenhancement endures, Pancrazio noted that the risks of brain-machine interfaces are not insignificant,

even for some of the devices currently available. For deep-brain stimulation, there is a 5% risk of infection, he said, and a 2% risk of stroke due to bleeding in the brain.

Nonetheless, neurostimulation devices “are a clinical reality for a large number of individuals who are living with disability,” Pancrazio said, and their benefits “truly are life-restoring for many individuals.” They also are a big business (a \$2.6 billion market in 2012, according to one study), and new devices continue to emerge.

Pancrazio cited an estimate that there are now more than 700,000 stimulation devices in use worldwide for various neurological conditions, including spinal cord stimulators

for chronic pain; sacral nerve stimulators for urinary incontinence; deep-brain stimulators for essential tremor, dystonia, and Parkinson’s disease; and cochlear implants for hearing loss.

In February, the U.S. Food and Drug Administration approved the Argus II bionic eye for “humanitarian use” in treatment of retinitis pigmentosa, an inherited, degenerative eye disease that often results in profound vision loss. The device, available in Europe since 2011, converts video images captured by a miniature camera, housed in the patient’s glasses, into electrical pulses that are transmitted wirelessly to an electrode array on the surface of the retina. These pulses stimulate the remaining retinal cells, producing light patterns in the patient’s field of view.

Ramez Naam, a computer scientist and author of *More than Human: Embracing the Promise of Biological Enhancement*, noted that researchers also have made strides getting data out of the brain via interfaces that allow, for example, a paralyzed patient to move a prosthetic hand just by “thinking” how it should move.

“In my opinion, the most powerful impact these technologies could have someday, probably decades and decades in the future, is by enhancing the ability of humans to communicate,” said Naam, who titled his talk “Networked Minds.” He described an experiment at Wake Forest University in which two capuchin monkeys in separate rooms were linked by an interface implanted in the auditory cortices of their brains. When a sound was played for one animal, the other was able to recognize it.

In another recent experiment, a researcher at the University of Washington, wired up to an electroencephalography machine, sent a brain signal via the Internet to a colleague across campus, causing the colleague’s right index finger to move on a keyboard. He wore a cap with a transcranial magnetic stimulation coil that sent the signal to the brain region that controls movement of the right hand.

While there are very real issues about the cost and control of technologies capable of human enhancement, Naam said, “I am an optimist. The long-term trajectory of information technology, which is how I view this in a way, is positive.”

—Earl Lane



Cross-campus communication. Rajesh Rao (left) at the University of Washington sends a brain signal to Andrea Stocco, causing Stocco’s finger to move.

conditions. Over the longer term, they said, any efforts to move beyond therapy to “enhancement” must weigh legal, ethical, and social concerns as well as the medical risks and benefits.

“If we are capable of enhancing, should we and how?” asked Daofen Chen, program director in systems and cognitive neuroscience at the National Institute of Neurological Disorders and Stroke. “Is it desirable?” There already have been efforts to enhance learning with prescription stimulants dubbed “smart” pills, but Chen said that there is no evidence that such pills truly improve cognitive abilities through a known biological mechanism.

Experts Warn Against Bans on 3D Printing

Last spring, news that a functioning plastic handgun had been created using a 3D printer, and that the file for making the gun was available online, led several city and state legislators to introduce bills banning 3D-printed guns.

However, 3D printers—which create customized objects on demand, based on digital instructions—are already widespread. Along with other “advanced additive manufacturing” technologies, these printers are used everywhere from large companies, to university labs, to home garages. Banning one type of 3D-printed object would be futile and could push 3D printing “underground,” at the expense of important scientific and medical advances, a panel of experts agreed at a AAAS event.

Many 3D printers work somewhat like inkjet printers do, depositing layers of material that can be plastic, metal, or even living cells. Since the first demonstration of this technology in the 1980s, numerous 3D printers have come on the market, and more should be showing up within the next year as the original patents expire. A relatively sophisticated, extrusion-based printer is about \$2500 to \$3000, and cruder models are available for as little as \$300 to \$400, according to Michael Hopmeier, President of Unconventional Concepts, Inc.

Those who don't own a printer can design and order items from a company like Shapeways, which fabricates and ships objects based on customers' specifications. The company is now producing about 50,000 objects each month, said Robert Schouwenburg, one of the company's cofounders.

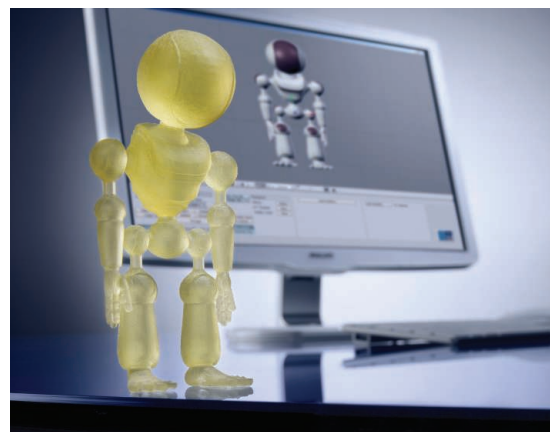
“3D printing is not just a device, it's an entire ecosystem of contributing technologies that give new capabilities,” said author and technology analyst Melba Kurman.

While the speakers at the 23 September event, which was organized by the AAAS Center for Science, Technology and Security Policy, agreed that banning 3D printed objects is unrealistic, they acknowledged that the technology gives rise to safety and security issues that merit serious thought.

3D printers have been used, for example, to create keys that can open master locks. In theory, they could also be used to build advanced weapons or to counterfeit machinery parts intended to break and cause catastrophic failure.

Some security solutions may come in the form of the technology itself. For example, Microsoft has demonstrated a method of embedding microbubbles within 3D-printed objects, in patterns that encode information. Such tags might potentially be used to trace the objects back to their source.

Although none of the proposals to ban 3D-printed guns ultimately became law, the speakers were concerned that similar, “fear-based” policy responses would stifle



Objects made to order. 3D printing is an important platform for innovation, and any regulatory responses should be based on sound science, panelists said.

the culture of openness necessary for this technology to thrive. Any regulatory decisions should be based on sound science and technology, they agreed.

Rob Carlson, a principal at the strategy and consulting firm Biodesic, has seen firsthand that some “garage biology,” which is responsible for increasing amounts of innovation in the life sciences, is already happening in secrecy so as to avoid harassment by law enforcement, even though the research being conducted is entirely legal. “That’s not the kind of world we want to create for biology or 3D printing,” he said. “We don’t want people to be afraid of talking about what they’re doing.”

AAAS Council Reminder

The next meeting of the AAAS Council will take place during the 2014 AAAS Annual Meeting in Chicago and will begin at 9:00 a.m. on 16 February 2014 in the Plaza Ballroom of the Hyatt Regency Chicago.

Individuals or organizations wishing to present proposals or resolutions for possible consideration by the council should submit them in written form to AAAS Chief Executive Officer Alan I. Leshner by 28 November 2013. This will allow time for them to be considered by the Committee on Council Affairs at its winter meeting.

Items should be consistent with AAAS's objectives and be appropriate for consideration by the council. Resolutions should be in the traditional format, beginning with “Whereas” statements and ending with “Therefore be it resolved.”

Late proposals or resolutions delivered to the AAAS Chief Executive Officer in advance of the February 2014 open hearing of the Committee on Council Affairs will be considered, provided that they deal with urgent matters and are accompanied by a written explanation of why they were not submitted by the November deadline. The Committee on Council Affairs will hold its open hearing at 2:30 p.m. on 15 February 2014 in the Hyatt Regency Plaza Ballroom.

Additional Candidates, AAAS General Election

The following candidates have been added to the general election ballot for the 2013 election of AAAS officers. The 2013 AAAS election of general and section officers is scheduled to begin in November. For a list of section candidates, please see AAAS News & Notes in the 27 September issue of *Science*.

General Election

President-Elect: Lance R. Collins, Cornell Univ.; Geraldine (Geri) Richmond, Univ. of Oregon

Board of Directors: Carlos J. Bustamante, Univ. of California, Berkeley; Sean R. Eddy, HHMI Janelia Farm Research Campus; Laura H. Greene, Univ. of Illinois at Urbana-Champaign; Joseph L. Travis, Florida State Univ.

Committee on Nominations: A. Paul Alivisatos, Univ. of California, Berkeley/Lawrence Berkeley National Laboratory; John E. Burris, Burroughs Wellcome Fund; Sylvia T. Ceyer, Massachusetts Institute of Technology; Christopher Bower Field, Carnegie Institution for Science/Stanford Univ.; Susan M. Fitzpatrick, James S. McDonnell Foundation; Alice P. Gast, Lehigh Univ.; Susan Gottesman, National Cancer Institute/NIH; Thomas Dean Pollard, Yale Univ.

Fine Tuning of Craniofacial Morphology by Distant-Acting Enhancers

Catia Attanasio, Alex S. Nord, Yiwen Zhu, Matthew J. Blow, Zirong Li, Denise K. Liberton, Harris Morrison, Ingrid Plajzer-Frick, Amy Holt, Roya Hosseini, Sengthavy Phouanavong, Jennifer A. Akiyama, Malak Shoukry, Veena Afzal, Edward M. Rubin, David R. FitzPatrick, Bing Ren, Benedikt Hallgrímsson, Len A. Pennacchio, Axel Visel*

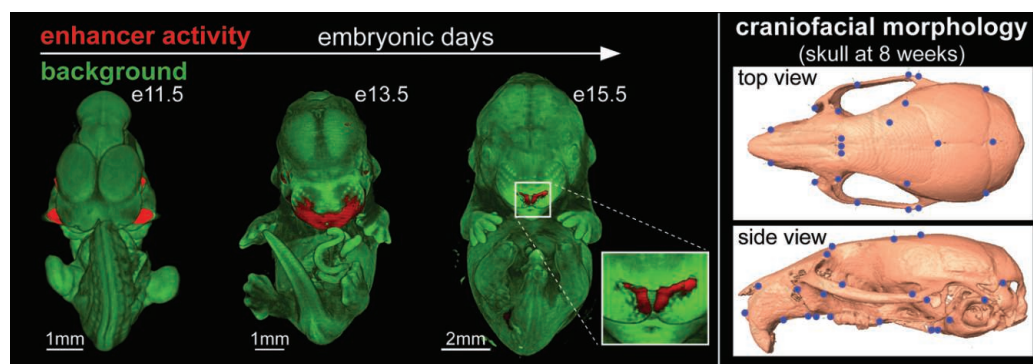
Introduction: The shape of the face is one of the most distinctive features among humans, and differences in facial morphology have substantial implications in areas such as social interaction, psychology, forensics, and clinical genetics. Craniofacial shape is highly heritable, including the normal spectrum of morphological variation as well as susceptibility to major craniofacial birth defects. In this study, we explored the role of transcriptional enhancers in the development of the craniofacial complex. Our study is based on the rationale that such enhancers, which can be hundreds of kilobases away from their target genes, regulate the spatial patterns, levels, and timing of gene expression in normal development.

Methods: To identify distant-acting enhancers active during craniofacial development, we used chromatin immunoprecipitation on embryonic mouse face tissue followed by sequencing to identify noncoding genome regions bound by the enhancer-associated p300 protein. We used LacZ reporter assays in transgenic mice and optical projection tomography (OPT) to determine three-dimensional expression patterns of a subset of these candidate enhancers. Last, we deleted three of the craniofacial enhancers from the mouse genome to assess their effect on gene expression and craniofacial morphology during development.

Results: We identified more than 4000 candidate enhancer sequences predicted to be active in the developing craniofacial complex. The majority of these sequences are at least partially conserved between humans and mice, and many are located in chromosomal regions associated with normal facial morphology or craniofacial birth defects. Characterization of more than 200 candidate enhancer sequences in transgenic mice revealed a remarkable spatial complexity of *in vivo* expression patterns. Targeted deletions of three craniofacial enhancers near genes with known roles in craniofacial development resulted in changes of expression of those genes as well as quantitatively subtle but definable alterations of craniofacial shape.

Discussion: Our analysis identifies enhancers that fine tune expression of genes during craniofacial development in mice. These results support that variation in the sequence or copy number of craniofacial enhancers may contribute to the spectrum of facial variation we find in human populations. Because many craniofacial enhancers are located in genome regions associated with craniofacial birth defects, such as clefts of the lip and palate, our results also offer a starting point for exploring the contribution of noncoding sequences to these disorders.

Craniofacial developmental enhancers contribute to craniofacial morphology. We identified distant-acting transcriptional enhancers active in the developing craniofacial complex and studied their activity patterns in detail in transgenic mice (**left**). Selected enhancers were deleted from the genome in mice in order to examine their role in modulating craniofacial morphology, which revealed subtle but significant effects of enhancers on the shape of the face and skull (**right**).



READ THE FULL ARTICLE ONLINE
<http://dx.doi.org/10.1126/science.1241006>



Cite this article as C. Attanasio *et al.*,
Science **342**, 1241006 (2013).
 DOI: 10.1126/science.1241006

FIGURES IN THE FULL ARTICLE

Fig. 1. Study overview.

Fig. 2. Genome-wide identification of candidate craniofacial enhancers.

Fig. 3. Transgenic characterization of craniofacial candidate enhancers results in the identification of facial substructure-specific enhancers.

Fig. 4. Regulatory landscapes of craniofacial loci.

Fig. 5. Developmental activity patterns of three enhancers selected for deletion studies.

Fig. 6. Expression phenotypes resulting from craniofacial enhancer deletions.

Fig. 7. Enhancer deletions cause changes of craniofacial morphology.

SUPPLEMENTARY MATERIALS

Material and Methods
 Supplementary Text
 Figs. S1 to S6
 Tables S1 to S6
 Movies S1 to S20
 References

ADDITIONAL RESOURCES

Genomic data sets, transgenic reporter data, and optical projection tomography data for craniofacial enhancers can be found in the Vista Enhancer Browser (<http://enhancer.lbl.gov>) and the FaceBase database (<http://facebase.org>) under the identifiers provided throughout the manuscript.

The list of author affiliations is available in the full article online.

*Corresponding author. E-mail: avisel@lbl.gov

Fine Tuning of Craniofacial Morphology by Distant-Acting Enhancers

Catia Attanasio,¹ Alex S. Nord,¹ Yiwen Zhu,¹ Matthew J. Blow,² Zirong Li,^{3*} Denise K. Liberton,⁴ Harris Morrison,⁵ Ingrid Plajzer-Frick,¹ Amy Holt,¹ Roya Hosseini,¹ Sengthavy Phouanavong,¹ Jennifer A. Akiyama,¹ Malak Shoukry,¹ Veena Afzal,¹ Edward M. Rubin,^{1,2} David R. FitzPatrick,^{5,6} Bing Ren,³ Benedikt Hallgrímsson,^{4,7} Len A. Pennacchio,^{1,2} Axel Visel^{1,2,†}

The shape of the human face and skull is largely genetically determined. However, the genomic basis of craniofacial morphology is incompletely understood and hypothesized to involve protein-coding genes, as well as gene regulatory sequences. We used a combination of epigenomic profiling, *in vivo* characterization of candidate enhancer sequences in transgenic mice, and targeted deletion experiments to examine the role of distant-acting enhancers in craniofacial development. We identified complex regulatory landscapes consisting of enhancers that drive spatially complex developmental expression patterns. Analysis of mouse lines in which individual craniofacial enhancers had been deleted revealed significant alterations of craniofacial shape, demonstrating the functional importance of enhancers in defining face and skull morphology. These results demonstrate that enhancers are involved in craniofacial development and suggest that enhancer sequence variation contributes to the diversity of human facial morphology.

The shape of the face is one of the features that most distinguishes individual humans from one another. Differences in facial morphology have substantial implications in many areas, including social interaction, psychology, forensics, and clinical genetics (1–3). The resemblance of facial shapes within families in general, and between monozygotic twins in particular, suggests a major contribution of genetic factors to craniofacial morphology (4–6). Many protein-coding genes whose disruption causes major aberrations of craniofacial morphology are known. This includes pathological dysmorphologies of the face itself, such as clefts of the lip or palate, as well as distinctive facial features associated with genetic syndromes that are indicative of associated pathologies in other organ systems (7–14). In contrast to these disease-related genes, only a small number of candidate genes have been implicated in normal variation of craniofacial shape through genome-wide association studies, and collectively they explain only a minute fraction of the morphological variation observed in human populations (15–17). We are interested

to understand how complex traits such as the individualized shape of the face can be modulated in subtle ways while avoiding the often severe consequences associated with protein-coding mutations (18).

Recent observations of large numbers of distant-acting transcriptional enhancers in mammalian genomes (19–21) raise the possibility that these sequences regulate development of structures such as the craniofacial complex. Enhancers can be located hundreds of kilobases away from their target genes and typically have highly restricted *in vivo* activity patterns. They often control the expression of their target genes in a modular fashion, where different enhancers activate the

expression of the same gene in different cell types, anatomical regions, or at different developmental time points. In principle, such complex arrays of enhancers acting on individual genes may provide a general mechanism for the independent fine-tuning of distinct aspects of gene expression in different developmental processes, which in turn may affect specific phenotypic traits, including facial shape (22). This model is consistent with the extensive studies of the genes and gene regulatory networks involved in the development of the neural crest, a cell population contributing to multiple tissues, including facial bone and cartilage (23). In-depth studies of individual genes involved in neural crest development [e.g., (24–26)], as well as genome-wide studies of regulatory sequences active in human neural crest cells (27), support that many genes involved in craniofacial development are associated with complex regulatory architecture. We used chromatin immunoprecipitation on whole face tissue to explore the genome-wide landscape of craniofacial enhancers in mice, and we studied enhancer involvement in defining mouse craniofacial morphology using transgenic reporter assays and enhancer knockout studies.

Identification of *In Vivo* Craniofacial Enhancers

To identify craniofacial developmental enhancers on a genome-wide scale, we performed chromatin immunoprecipitation followed by sequencing (ChIP-Seq) analysis on mouse embryonic-day 11.5 (e11.5) facial tissue with the enhancer-associated p300 protein (Fig. 1) (21). At this developmental time point, key events of craniofacial development are in progress, including growth and morphogenetic processes affecting the size, shape, and structure of all major craniofacial prominences (28, 29). All major facial subregions were included in this tissue preparation (30), building on the previously

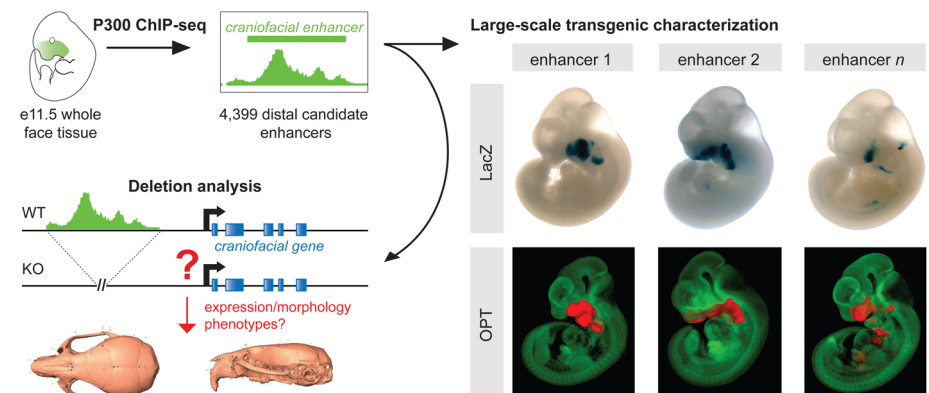


Fig. 1. Study overview. We performed p300 ChIP-Seq on whole mouse face tissue from e11.5 embryos, which identified 4399 putative distant-acting craniofacial enhancers. More than 200 craniofacial candidate enhancers were characterized in depth through LacZ transgenesis in mouse embryos (LacZ; top right), and selected enhancers were further analyzed by means of optical projection tomography (OPT; bottom right). Unstained tissue is shown in green, and LacZ-stained tissue is shown in red. The examples shown here are enhancers mm622, mm924, and mm613. Furthermore, a panel of three enhancers near functionally unrelated genes was studied by means of knockout analysis and detailed skull morphometry in mice. Blue dots indicate standardized morphometric landmarks.

¹Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA. ²U.S. Department of Energy Joint Genome Institute, Walnut Creek, CA 94598, USA. ³Ludwig Institute for Cancer Research, and Department of Cellular and Molecular Medicine, University of California, San Diego School of Medicine, 9500 Gilman Drive, La Jolla, CA 92093, USA. ⁴Department of Cell Biology and Anatomy, McCaig Bone and Joint Institute, University of Calgary, Calgary T2N 4N1, Canada. ⁵MRC Human Genetics Unit, MRC Institute for Genetic and Molecular Medicine, University of Edinburgh, Edinburgh EH4 2XU, UK. ⁶Royal Hospital for Sick Children, Edinburgh EH9 1LF, UK. ⁷Alberta Children's Hospital Research Institute, University of Calgary, Calgary T2N 4N1, Canada.

*Present address: EMD Millipore, 28820 Single Oak Drive, Temecula, CA 92590, USA.

†Corresponding author. E-mail: avisel@lbl.gov

described efficiency of this inclusive approach to identify enhancers with both broad and tightly confined patterns in subregions of developing embryonic structures (31, 32).

Enrichment analysis identified 4399 distal candidate enhancers genome-wide, defined as regions that showed significant p300 binding in craniofacial tissue and were at least 2.5 kb from known transcription start sites (Fig. 2 and tables S1 and S2). Candidate enhancers were located up to 1.4 Mb (median distance, 44 kb) from the nearest known transcript start site, with 38.4% in introns of genes and 54.7% located in noncoding regions outside of genes (intergenic). The majority of candidate enhancers also showed evidence of evolutionary constraint (87.5%) (table S1) and had unique orthologous sequences in the human genome (96.7%). Unbiased ontology analysis (33) revealed that candidate craniofacial enhancers are enriched near genes that are known to cause craniofacial phenotypes when deleted in mouse models or mutated in humans (table 1). Candidate craniofacial enhancers were also enriched at loci implicated in human craniofacial traits and birth defects through genome-wide association studies (fig. S1). These observations are consistent with a role of the identified enhancer candidate sequences in the regulation of genes with known roles in craniofacial development. Taken together, these results suggest that thousands of distant-acting enhancers are involved in orchestrating the genome-wide gene expression landscape during craniofacial development.

Large-Scale Transgenic Analysis of Craniofacial Enhancers

ChIP-Seq performed directly on craniofacial tissues provided a genome-wide catalog of sequences that are likely to be active in vivo enhancers during craniofacial development at e11.5. However, this approach does not provide direct insight into the exact activity patterns of individual candidate enhancer sequences. To examine craniofacial enhancer activity patterns in detail, we used transgenic enhancer reporter assays in mice, coupled to high-resolution three-dimensional (3D) mapping of LacZ reporter activities by means of optical projection tomography (OPT) (Fig. 1) (30, 34, 35). Because many, but not all, in vivo enhancers can be identified through p300 binding (36), we also considered sequence conservation (34) and proximity to genes or loci with a known role in craniofacial development as additional criteria in the selection of candidate sequences. In total, we tested 205 candidate sequences in transgenic mice, with the majority (123, or 60%) located within or near regions associated with craniofacial development through experimental, genetic, or genome-wide association studies (properties of all tested candidate sequences are provided in table S3). Each candidate enhancer sequence was coupled to a minimal promoter and used to generate multiple transgenic embryos by means of pronuclear injection (30). Only patterns that were independently observed in at least three different embryos were



Fig. 2. Genome-wide identification of candidate craniofacial enhancers. Mouse genome graph showing all p300-enriched regions (green dots) and all 281 sequences tested in vivo or reexamined for craniofacial activity in this study (red dots). Examples of selected major craniofacial genes (55) and genomic regions [such as regions orthologous to human 8q24 (43) and ABCA4 (46)] are highlighted by pink boxes. Known craniofacial loci were generally enriched in candidate sequences and were specifically targeted for sampling in transgenic assays (red dots). The three genomic regions studied by means of knockout analysis are highlighted by blue boxes.

Table 1. Top enriched annotations of mouse and human phenotypes associated with candidate craniofacial enhancers. (Top) Ten of the 12 most significantly enriched terms from the mouse phenotype ontology directly relate to craniofacial development. The remaining two phenotypes (abnormal axial skeleton morphology and abnormal skeleton development) relate to general skeleton development, a process that shares key signaling pathways with cranial skeleton development (58). (Bottom) Six of the 10 most significantly enriched terms from the human phenotype ontology are relevant to craniofacial development. The four remaining phenotypes are all associated with limb abnormalities, which is consistent with previous knowledge of shared developmental pathways during limb and face development (59–61). In each analysis, only terms exceeding twofold binomial enrichment were considered and ranked by *P* value (binomial raw *P* values).

Rank	Phenotype term	Binomial <i>P</i> value	Binomial fold enrichment
Mouse phenotypes			
1	Abnormal craniofacial morphology	5.8×10^{-110}	2.0
3	Abnormal head morphology	1.78×10^{-88}	2.1
4	Abnormal craniofacial development	3.88×10^{-82}	2.4
5	Abnormal craniofacial bone morphology	1.38×10^{-78}	2.1
6	Abnormal facial morphology	5.58×10^{-78}	2.2
7	Abnormal cranium morphology	3.18×10^{-77}	2.2
9	Abnormal mouth morphology	3.58×10^{-72}	2.3
10	Abnormal orofacial morphology	1.5×10^{-71}	2.3
11	Abnormal viscerocranium morphology	1.0×10^{-62}	2.3
12	Abnormal neurocranium morphology	2.1×10^{-60}	2.5
Human phenotypes			
2	Malar hypoplasia	3.6×10^{-17}	2.4
3	Abnormality of the midface	7.6×10^{-17}	2.3
5	Abnormal location of ears	5.7×10^{-16}	2.1
7	Low-set ears	1.1×10^{-15}	2.1
8	Abnormality of the fontanelles and cranial sutures	1.2×10^{-15}	2.2
9	Abnormality of the calvarium	1.3×10^{-15}	2.1

considered reproducible. In total, 121 of 205 tested sequences showed reproducible reporter gene expression in at least one craniofacial structure. We further extended the set of *in vivo*-characterized craniofacial enhancers by reexamining data from previously described large-scale enhancer screens not specifically targeted at craniofacial enhancer discovery (21, 31, 32, 34, 37–39), providing an additional 75 craniofacial enhancers (table S3). Transgenic results for the 196 craniofacial enhancers identified or reexamined in this study are available through the Vista Enhancer Browser (<http://enhancer.lbl.gov>) or the National Institute of Dental and Craniofacial Research (NIDCR) FaceBase consortium web site (<http://facebase.org>) (40).

To gain higher-resolution insight into the 3D activity patterns of craniofacial enhancers in the context of developing embryos, we used optical projection tomography (OPT). In total, representative embryos for 55 craniofacial enhancers, including 48 from this study, were analyzed with OPT. Selected examples of 3D views are provided as supplementary movies (movies S1 to

S11). More comprehensive OPT data collections can be interactively explored through a dedicated viewer at the NIDCR FaceBase database (fig. S2) (40). Examination of this large set of *in vivo*-validated and -characterized craniofacial enhancers highlights several salient features and resulting potential applications of these data sets, which we will describe using selected examples. Specifically, this collection of enhancers (i) highlights the diversity of enhancer activity patterns and the regulatory complexity of the genetic code, (ii) enables the dissection of the regulatory landscapes of individual genes known to be involved in craniofacial development, and (iii) provides a starting point for the mechanistic exploration of genomic intervals implicated in craniofacial development through genome-wide association studies.

Diversity of Patterns

To illustrate the reproducibility and diversity of craniofacial activity patterns identified in transgenic embryos, selected examples of enhancers identified in this study are shown in Fig. 3A.

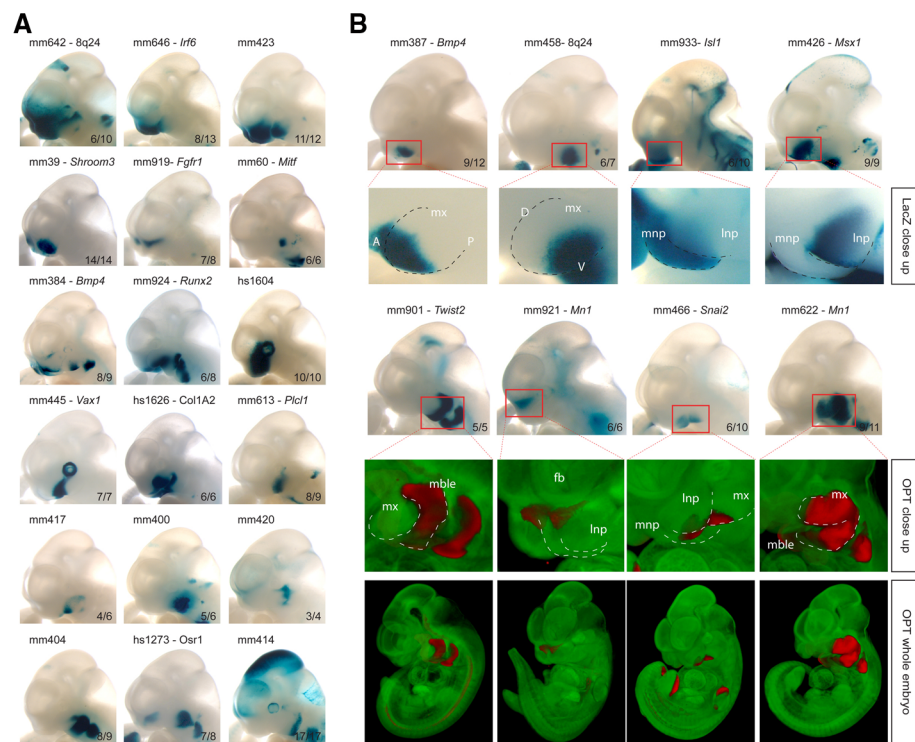


Fig. 3. Transgenic characterization of craniofacial candidate enhancers results in the identification of facial substructure-specific enhancers. (A) Selection of 18 reproducible craniofacial enhancers at e11.5 illustrates the broad spectrum of activity patterns observed *in vivo*. For each tested candidate enhancer, one representative embryo face is shown; the reproducibility of each pattern among multiple transgenic founder embryos is indicated at the right bottom corner of each image. For each element, the nearest relevant craniofacial gene, if any, is also provided. Additional embryo images obtained with each enhancer construct can be viewed at <http://enhancer.lbl.gov> or <http://facebase.org>. (B) (Top) Four examples of highly restricted specificity to craniofacial substructures. (Bottom) Four examples of internal enhancer activity captured with OPT scanning of LacZ-stained embryos. Green indicates no LacZ activity (enhancer inactive), and red indicates LacZ activity (enhancer active). Embryos have an average crown-rump length of 6 mm. A, anterior; D, dorsal; fb, forebrain; lnp, lateral nasal prominence; mble, mandibular process; mnp, medial nasal prominence; mx, maxillary process; P, posterior; and V, ventral.

For all craniofacial prominences (medial nasal, lateral nasal, maxillary, and mandibular), structure-specific active enhancers were identified (Fig. 3A; a schematic view of the e11.5 mouse face is provided in fig. S4A). In depth analysis of craniofacial activity patterns through the combined use of whole-mount LacZ staining and OPT imaging revealed that in many cases only subregions of these structures were reproducibly targeted by an enhancer. For example, enhancer mm387 drives expression in the anterior part of the maxillary prominence, whereas enhancer mm458 is restricted to a posterior ventral region (Fig. 3B, top). Similar region-specific activities are observed in other facial substructures—such as the nose, where enhancer mm933 is active in the medial nasal prominence, whereas the activity of enhancer mm426 is confined to the lateral nasal prominence (Fig. 3B, top). OPT scans of whole-mount embryos provide additional spatial information about enhancer activity pattern by capturing the activity signal in internal embryonic structures (Fig. 3B, bottom). These data highlight the complexity, diversity, and spatially highly restricted activity patterns of distant-acting enhancer sequences active during craniofacial development.

Regulatory Landscapes of Craniofacial Genes

Systematic screening of individual genomic loci via ChIP-Seq followed by transgenic characterization enables functional dissection of the distant-acting enhancer landscapes of individual genes with known roles in craniofacial development. As an example, mouse *Msx1* and human *MSX1* have been studied for their role in craniofacial development (supplementary text) (41). *Msx1* is surrounded by several hundred kilobases of noncoding DNA, which renders the search for distant-acting enhancers challenging. Transgenic testing of seven candidate sequences identified with ChIP-Seq and located up to 235 kb away from the *Msx1* transcription start site resulted in the identification of five distinct craniofacial enhancers potentially regulating its expression (Fig. 4A). At e11.5, each of these enhancers drove patterns that partially recapitulated the endogenous *Msx1* RNA expression. For instance, *Msx1* activity in the second branchial arch and in the maxillary process of the e11.5 embryo is recapitulated by the combined activity of two separate enhancers located at 1 and 235 kb upstream of the promoter (mm426 and hs746) (Fig. 4A). These observations support the notion that complex spatial expression patterns of key developmental genes are driven by modular arrays of distant-acting enhancers (42) and highlights the potential of enhancers to provide a mechanism for fine tuning of *in vivo* gene expression patterns.

Craniofacial Enhancers Within Disease-Associated Intervals

To illustrate the utility of these enhancer data sets in the follow-up of genome-wide association, population-scale sequencing, and candidate locus studies, 50 candidate enhancers mapping to

intervals implicated in craniofacial morphology or orofacial birth defects through human genetic studies were included in the transgenic assays (table S3). Trait-associated variants that map to noncoding genome regions or are not linked to any protein-altering variants are a common challenge in the interpretation of such genetic studies. A prototypical example is a region of human chromosome 8q24 that is devoid of protein-coding genes. A 640-kb stretch located within this region is a major susceptibility locus for cleft palate, with a calculated population attributable risk of 41% (43–45). Variants at this locus are also significantly linked to normal variation in several facial morphology traits (16). We identified four craniofacial enhancer candidate sequences in the mouse genome region orthologous to the human risk interval, two of which drive reproducible craniofacial reporter activity at e11.5 in transgenic mice (Fig. 4B). As a second example, we examined the 1p22 locus. In this interval, markers located near and within the *ABCA4* gene are associated with an increased risk for cleft palate in humans, but it remains unclear whether these variants are linked to deleterious protein-coding mutations of *ABCA4* (46, 47). On the basis of RNA expression data, the neighboring gene *ARHGAP29*, rather than *ABCA4* itself, has been proposed to be causatively involved in craniofacial development (48). However, *ARHGAP29* falls outside the genomic boundaries of the risk-associated linkage block. By scanning the region comprising these two genes for possible associated enhancers, we identified a human-mouse conserved sequence in the first intron of *Abca4* that drove highly-reproducible reporter activity in the facial midline, a pattern reminiscent of *Arhgap29* RNA expression, suggesting that this enhancer may drive expression of *Arhgap29* during craniofacial development (Fig. 4C and movie S10) (49). A causative effect of sequence or copy number variants in these particular enhancers on craniofacial morphology remains to be demonstrated; furthermore, we cannot exclude the existence of additional enhancer sequences at these loci that were not captured in the present screen. These possible limitations notwithstanding, our results illustrate the utility of collections of validated enhancers as starting points for the mechanistic interpretation of human genetic studies by linking functional genomic and human genetic data sets.

Targeted Deletions of Craniofacial Enhancers

The existence of large numbers of distant-acting enhancers with precise tissue-specific activities during craniofacial development raises the question of their functional impact on craniofacial morphology through the regulation of their respective target genes. To examine such contributions in more detail, we selected three enhancers with highly reproducible craniofacial activity patterns and explored their functions through targeted deletions in mice (Fig. 1). The three enhancers—termed hs1431 (near *Snai2*), hs746

(near *Msx1*), and hs586 (near *Isl1*)—were chosen on the basis of their association with known craniofacial genes (supplementary text) (7, 50, 51), the robustness of their activity patterns, and the absence of additional known enhancers with overlapping activity near the same gene. Furthermore, the in vivo activity patterns driven by these enhancers partially recapitulate the known expression patterns of their presumptive target genes (Fig. 4A and fig. S3). The enhancers were in-

tentionally chosen from different, functionally unrelated loci in order to provide a representative sample of the genome-wide enhancer data set, rather than an in-depth exploration of a single gene or pathway. All selected enhancers are located at a very long distance from their respective target genes (350, 235, and 190 kb, respectively) and are active in the craniofacial complex through multiple stages of embryonic development (Figs. 4A and 5, fig. S3, and movies S1 to S9).

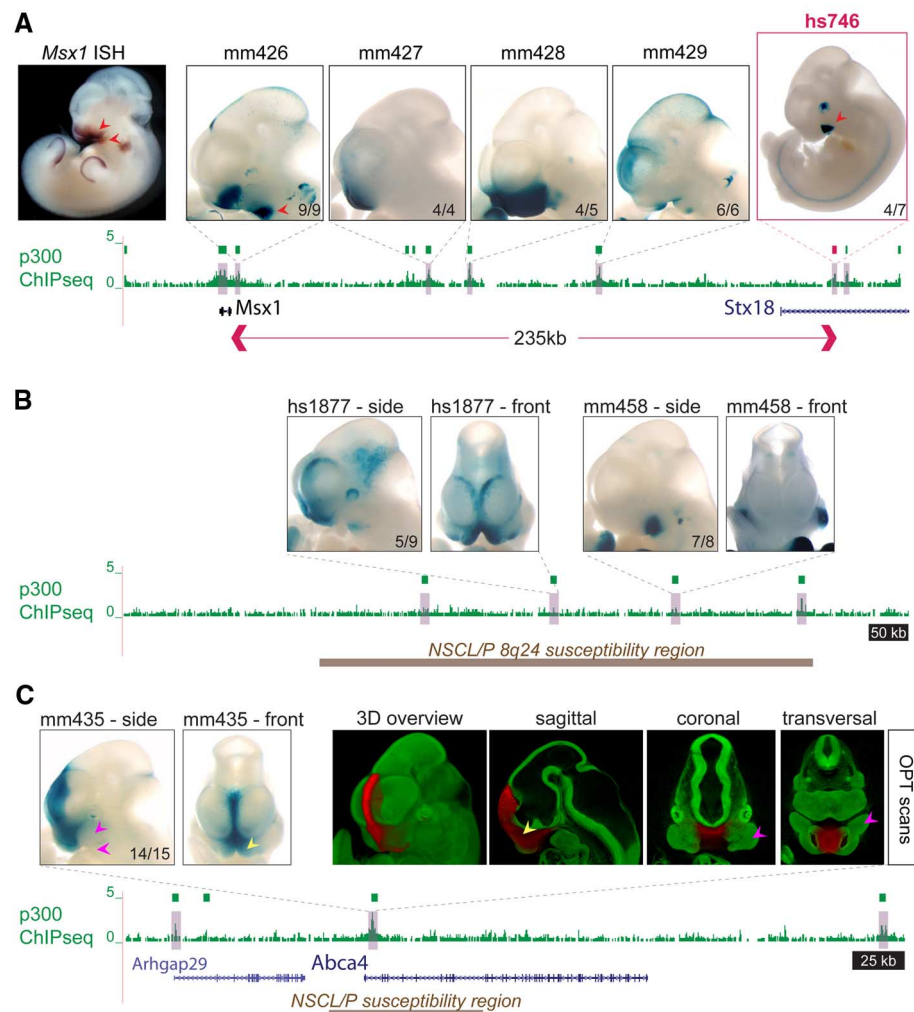


Fig. 4. Regulatory landscapes of craniofacial loci. (A) Craniofacial enhancers near *Msx1*, a major craniofacial gene, were identified with p300 ChIP-Seq (green boxes). This included the reidentification of a region proximal to *Msx1* with previously described enhancer activity (mm426) (56), as well as four additional, more distal enhancers with complementary activity patterns. For each enhancer, only one representative embryo is shown; numbers indicate reproducibility. Red arrows indicate selected correlations between *Msx1* RNA expression (ISH) and individual enhancers. Red box indicates enhancer hs746, which was further studied by means of knockout analysis. *Msx1* ISH is from Embryos database (<http://embryos.jp>) (57). (B) Identification of craniofacial enhancers in the left- and morphology-associated gene desert at human chromosome 8q24 (orthologous mouse region shown) (43). Brown box indicates the region corresponding to a 640-kb human region associated with orofacial clefts [nonsyndromic cleft lip with or without cleft palate (NSCL/P)] and devoid of protein-coding genes. Two of four candidate enhancers within the region drove craniofacial expression. For each enhancer, lateral and frontal views of one representative embryo are shown. (C) Identification of a craniofacial midline enhancer at the cleft-associated susceptibility interval at the *ABCA4* locus (46). The enhancer is highly active in the nasal prominences (yellow arrows), but not the maxillary or mandible (pink arrows). Embryos have an average crown-rump length of 6 mm.

To test whether these enhancers are important in modulating craniofacial morphology, we created three separate mouse lines carrying deletion alleles for each of the three enhancers using a standard homologous recombination strategy in embryonic stem cells (30). Mice homozygous for any of the three enhancer deletions do not display gross craniofacial malformations or other obvious deficiencies. To evaluate the effect of each enhancer deletion on the expression of the presumptive target genes (*Snai2*, *Msx1*, and *Isl1*), we used quantitative reverse transcription polymerase chain reaction to measure transcript levels in different craniofacial structures of individual wild-type and enhancer deletion embryos

(littermates) at e11.5 and e13.5 (Fig. 6 and fig. S4). Depending on time-point and substructure, we observed up to 3.9-fold down-regulation ($P = 4 \times 10^{-5}$) of *Snai2* in homozygous Δ hs1431 embryos, 1.5-fold down-regulation ($P = 0.015$) of *Msx1* in Δ hs746, and 1.3-fold down-regulation ($P = 0.04$) of *Isl1* in Δ hs586 (Fig. 6, C and D, and fig. S4E). In all cases, the changes in transcript levels of the respective target gene were confined to subregions in which the enhancer was active. However, not all subregions with enhancer reporter activity showed significant down-regulation of the target gene. These observations raise the possibility of partial functional redundancy between the enhancers studied here and

overlapping regulatory activities from gene promoters or additional distant-acting enhancers that were not captured in our genome-wide screen. Regardless of the presence of possible additional regulatory sequences in these genome intervals, these results provide evidence for the requirement of enhancers for normal gene expression during craniofacial development.

To examine whether the deletion of these enhancers altered craniofacial morphology, we compared mouse skulls from wild-type and enhancer deletion mice at 8 weeks of age. Because it is challenging to quantify possible differences in craniofacial morphology with visual observation alone, we used micro-computed tomography (micro-CT) to obtain accurate 3D measurements of the skulls. Three cohorts, each consisting of at least 30 mice homozygous for a deletion of one of the three enhancers, were compared with a cohort of 44 wild-type littermates. Micro-CT reconstructions of each mouse head were measured by using 54 standardized skeletal landmarks (fig. S5). The cohorts of wild-type and enhancer deletion mice were compared by using canonical variate analysis (CVA) to identify possible changes in craniofacial morphology resulting from the enhancer deletions (Fig. 7). Procrustes analysis of variance (ANOVA) ($F = 12.0$, $P < 0.0001$) and multivariate ANOVA (Pillai's Trace 2.5, $P < 0.0001$) tests both showed that enhancer deletion genotypes were significantly associated with alterations of craniofacial shape. All individual pair-wise permutation tests (Procrustes distances) between wild-type and enhancer deletion lines revealed significant differences (table S4), with the most pronounced differences observed for Δ hs1431 and Δ hs746 (both $P < 0.0001$ compared with wild-type). Differences between wild-type, Δ hs1431, and Δ hs746 mice were also significant after Bonferroni adjustment for the six pairwise comparisons between groups. The largest magnitude of effect on shape was observed for Δ hs1431, followed by an intermediate quantitative effect for Δ hs746 (Fig. 7B), whereas possible changes in Δ hs586 were not statistically significant after correction for multiple hypothesis testing. These results mirror the magnitude of expression phenotypes, which were most pronounced in Δ hs1431, followed by intermediate changes in Δ hs746 and only a limited expression phenotype observed in Δ hs586 (Fig. 6 and fig. S4). These results show that deletion of enhancers can affect craniofacial morphology.

Each enhancer deletion causes a distinct set of differences as compared with wild-type morphology. This is evident from the CVA, in which the first three canonical variates (CV1 to CV3) most clearly separate wild-type mice from Δ hs1431, Δ hs746, and Δ hs586, respectively (Fig. 7). Each enhancer deletion produces phenotypic effects that are not confined to a single feature but involve multiple regions of the skull (Fig. 7C and movies S12 to S20). For example, deletion of *hs1431* results in an increase in facial length, a relative increase in the width of the anterior

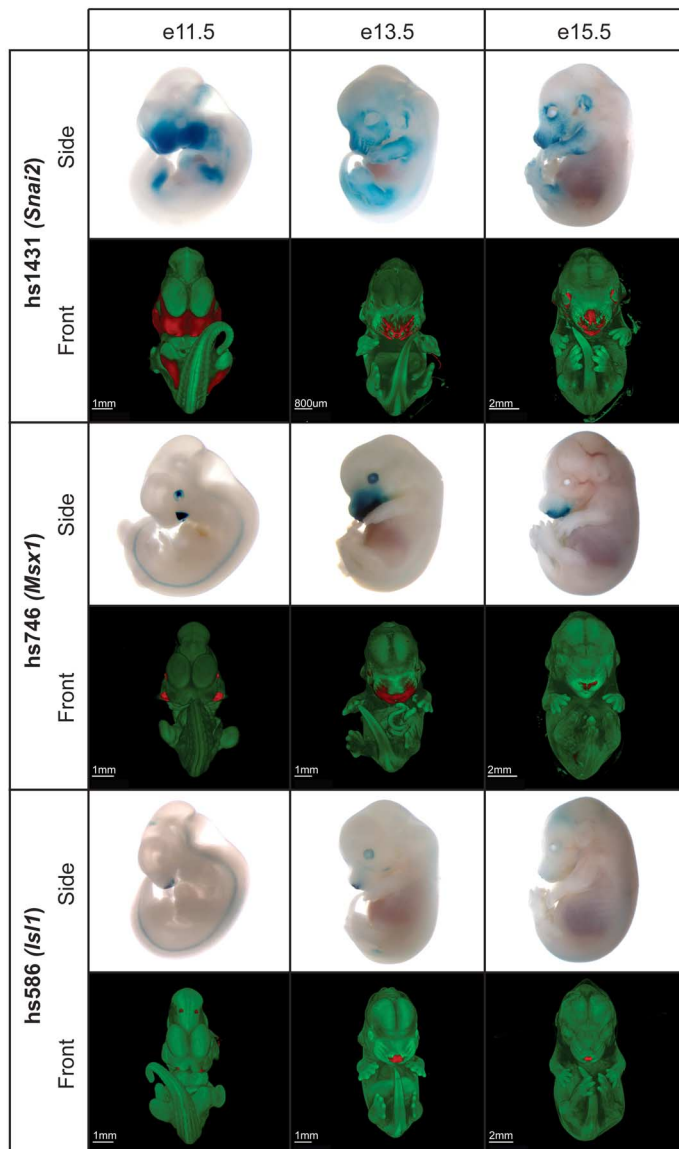


Fig. 5. Developmental activity patterns of three enhancers selected for deletion studies. The in vivo activity of each enhancer was monitored at different stages of development (e11.5, e13.5, and e15.5) (movies S1 to S9). All enhancers were reproducibly active in the craniofacial complex during embryonic development, with spatial changes in activity across stages. Side views are of LacZ-stained whole-mount embryos. Front views are optical projection tomography reconstructed 3D images. Regions of enhancer activity are shown in red.

neurocranium, and a shortening of the anterior cranial base. In contrast, Δ hs746 results in a shortening of the face, a widening of the posterior neurocranium, a narrowing of the palate, and shortening of the cranial base. Although both Δ hs1431 and Δ hs746 have significant effects on facial morphology in structures derived from regions with enhancer activity at e11.5 and e13.5 (Fig. 6), there are also changes in other parts of the skull. These correlated patterns of change are consistent with numerous studies demonstrating that cranium development is a highly integrated process and that variation of the skull is structured by complex interactions between the growing chondrocranium, neurocranium, and other nearby tissues (52, 53). Regardless of the precise molecular pathways and developmental mechanisms that underlie the morphological changes observed upon deletion of these enhancers, these results demonstrate that distant-acting enhancers contribute to the development of craniofacial shape in mammals. The observation of significant but nonpathological alterations of craniofacial morphology as a result of enhancer deletions supports the notion that enhancers contribute to normal variation in facial shape.

Conclusions

The general shape of the human face and skull, the differences in facial shape between individuals, and the high heritability of facial shape are subjects of broad interest because they have far-reaching implications well beyond basic scientific and biomedical considerations. In this study, we examined the possible impact of distant-acting regulatory sequences on craniofacial morphology. Throughout the genome, we identified several thousand sequences that are likely to be distant-acting enhancers active in vivo during mammalian craniofacial development. Although this epigenomic analysis was performed in the mouse, the vast majority of these enhancer candidate sequences are conserved between mouse and human. Large-scale characterization of more than 200 candidate sequences in transgenic mice showed the versatility of enhancers in orchestrating gene expression during craniofacial development. These observations are consistent with genome-wide analyses of enhancers active in human neural crest cells, as well as studies of regulatory sequences associated with individual members of the neural crest gene regulatory network (23–27). We also demonstrated that deletion of craniofacial enhancers results in nonpathological but measurable changes in craniofacial morphology in mice. Taken together, these data support that enhancers are involved in determining craniofacial shape. Systematic genome-wide studies of normal morphological variation in human populations are beginning to emerge (15–17) and will offer the opportunity to compare in vivo-derived genome-wide maps of craniofacial enhancers identified in this study with variation data in order to gain further mechanistic insight into

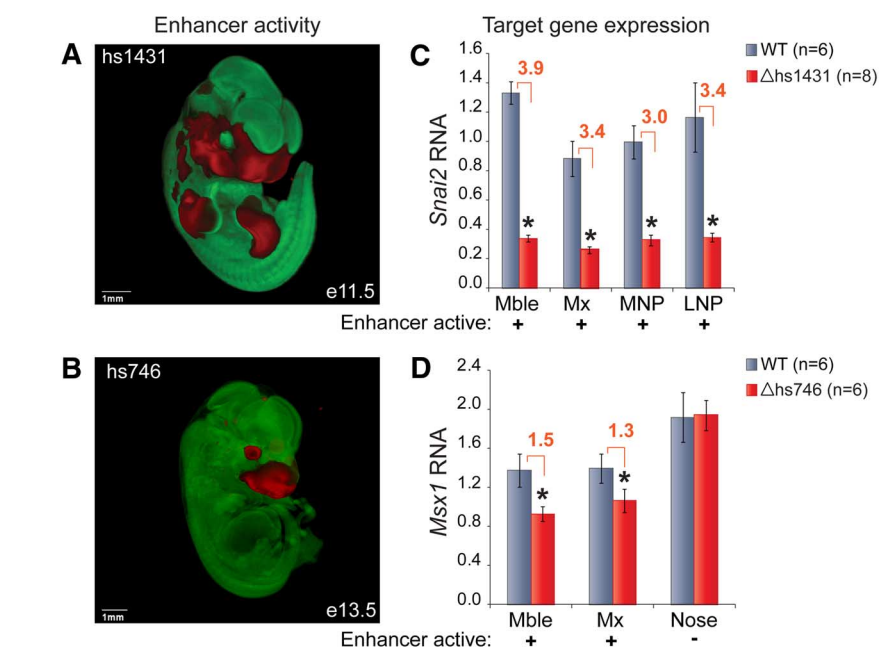


Fig. 6. Expression phenotypes resulting from craniofacial enhancer deletions. (A and B) In vivo activity pattern of hs1431 (at e11.5) and hs746 (at e13.5). OPT data are represented in red (LacZ, enhancer active) and green (no LacZ, enhancer inactive). (C and D) Expression levels of enhancer target genes in craniofacial tissues dissected from wild-type (gray) and knockout (red) littermate embryos. Error bars show the variation among individuals of the same genotype (SEM). * $P < 0.05$ (Student t test, one-tailed); Mble, mandibular; Mx, maxillary; MNP, medial nasal process; and LNP, lateral nasal process.

the molecular underpinnings of human facial shape and variation therein.

Beyond the spectrum of normal morphological variation in craniofacial shape, these results also provide a functional genomic framework for the analysis of craniofacial birth defects. We showed that deletion of craniofacial enhancers results in noticeable but nonpathological changes in morphology. Even for Δ hs1431, the enhancer deletion resulting in the most severe reduction in craniofacial gene expression, the morphological phenotype was overall much less severe than the pathological changes observed upon deletion of the *Snai2* gene itself (54). This milder phenotype is not surprising, considering that remaining baseline activity of the gene was observed in all craniofacial structures examined (Fig. 6A and fig. S4C). Although some enhancer deletions may lead to more severe phenotypes (26), these observations highlight the potential of enhancers to modulate craniofacial morphology in quantitatively subtle ways, without the pathological consequences potentially associated with deleterious protein-coding mutations. These results raise the possibility that sequence or copy number variation affecting more than one enhancer of the same gene may cumulatively result in more severe and potentially pathological phenotypes. Isolated examples of sequence variants in distant-acting enhancers associated with malformations such as clefts of the lip or palate have been described (49), and there is circumstantial evidence that noncoding sequences, including enhancers, contribute substantially to these processes (43). There

is partial overlap between loci involved in normal facial shape variation and in craniofacial birth defects, supporting the possibility that some dysmorphologies represent the extreme ends of the normal spectrum of variation (15, 16). The improved genome-wide functional annotation of craniofacial in vivo enhancers obtained through this study is expected to aid not only in the functional exploration of isolated studies of craniofacial dysmorphologies but may also facilitate an understanding of the links between normal and pathological variation in craniofacial shape.

References and Notes

1. K. Christensen, K. Juel, A. M. Herskind, J. C. Murray, Long term follow up study of survival associated with cleft lip and palate at birth. *BMJ* 328, 1405 (2004). doi: [10.1136/bmj.38106.559120.7C](https://doi.org/10.1136/bmj.38106.559120.7C); pmid: 15145797
2. G. L. Wehby, C. H. Cassell, The impact of orofacial clefts on quality of life and healthcare use and costs. *Oral Dis.* 16, 3–10 (2010). doi: [10.1111/j.1601-0825.2009.01588.x](https://doi.org/10.1111/j.1601-0825.2009.01588.x); pmid: 19656316
3. M. Kayser, P. M. Schneider, DNA-based prediction of human externally visible characteristics in forensics: Motivations, scientific challenges, and ethical considerations. *Forensic Sci. Int. Genet.* 3, 154–161 (2009). doi: [10.1016/j.fsigen.2009.01.012](https://doi.org/10.1016/j.fsigen.2009.01.012); pmid: 19414162
4. L. A. P. Kohn, The role of genetics in craniofacial morphology and growth. *Annu. Rev. Anthropol.* 20, 261–278 (1991). doi: [10.1146/annurev.an.20.100191.001401](https://doi.org/10.1146/annurev.an.20.100191.001401)
5. C. Manfredi, R. Martina, G. B. Grossi, M. Giuliani, Heritability of 39 orthodontic cephalometric parameters on MZ, DZ twins and MN-paired singletons. *Am. J. Orthod. Dentofacial Orthop.* 111, 44–51 (1997). doi: [10.1016/S0889-5406\(97\)70301-9](https://doi.org/10.1016/S0889-5406(97)70301-9); pmid: 9009923
6. B. Johannsdottir, F. Thorarinnsson, A. Thordarson, T. E. Magnusson, Heritability of craniofacial characteristics between parents and offspring estimated from lateral cephalograms. *Am. J. Orthod. Dentofacial*

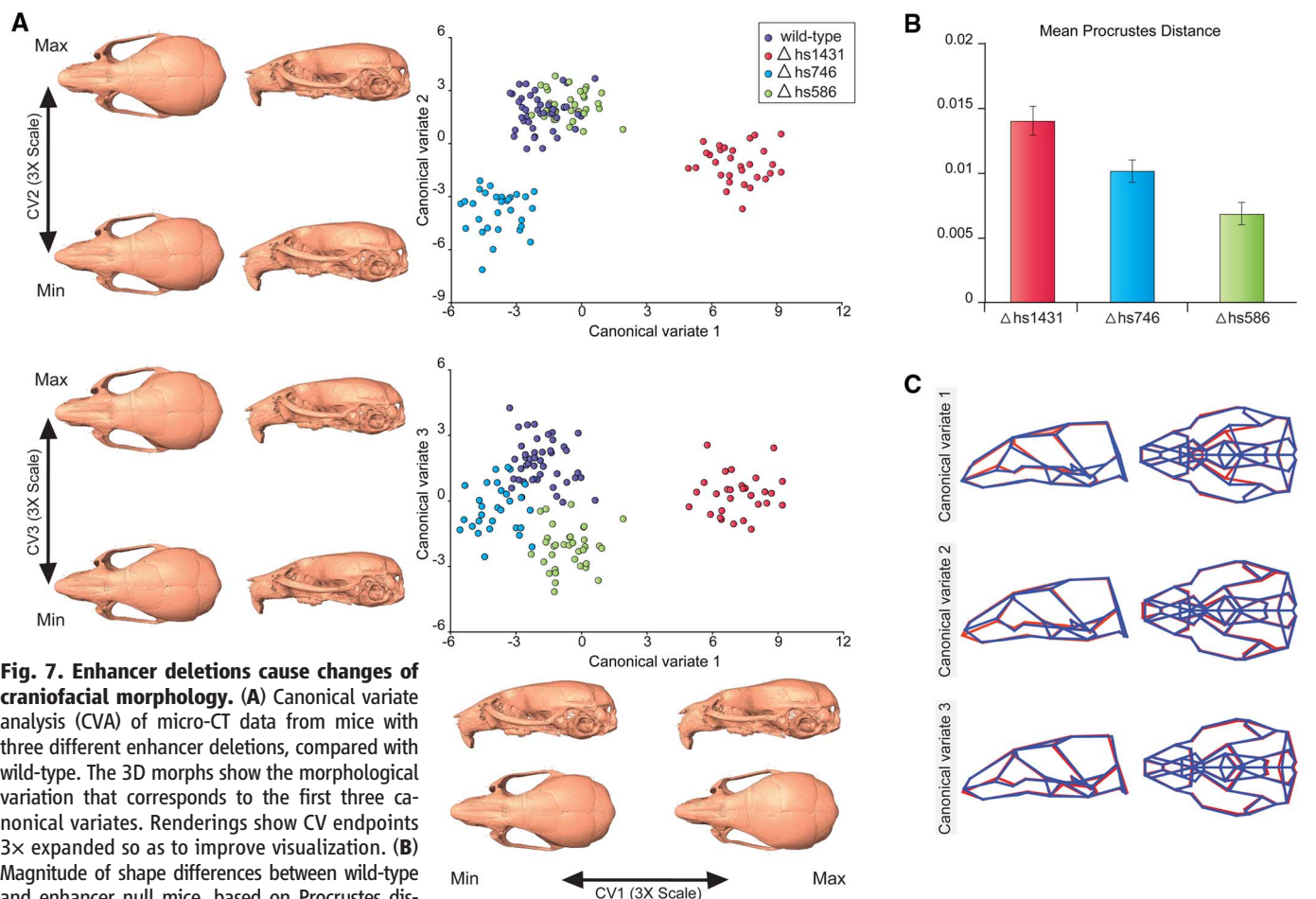


Fig. 7. Enhancer deletions cause changes of craniofacial morphology. (A) Canonical variate analysis (CVA) of micro-CT data from mice with three different enhancer deletions, compared with wild-type. The 3D morphs show the morphological variation that corresponds to the first three canonical variates. Renderings show CV endpoints 3× expanded so as to improve visualization. (B) Magnitude of shape differences between wild-type and enhancer null mice, based on Procrustes distances (30). Error bars indicate SD of shape differences from resampling Procrustes distances across 10,000 iterations. (C) Wireframe visualization of the first three canonical variates, which are predominantly driven by morphological differences between wild-type mice and Δ hs1431, Δ hs746, and Δ hs586, respectively. CV endpoints are superimposed as red and blue wireframes, respectively.

- Orthop.* **127**, 200–207, quiz 260–261 (2005). doi: [10.1016/j.ajodo.2004.07.033](https://doi.org/10.1016/j.ajodo.2004.07.033); pmid: [15750539](https://pubmed.ncbi.nlm.nih.gov/15750539/)
7. I. Satokata, R. Maas, *Mx1* deficient mice exhibit cleft palate and abnormalities of craniofacial and tooth development. *Nat. Genet.* **6**, 348–356 (1994). doi: [10.1038/ng0494-348](https://doi.org/10.1038/ng0494-348); pmid: [7914451](https://pubmed.ncbi.nlm.nih.gov/7914451/)
 8. M. J. van den Boogaard, M. Dorland, F. A. Beemer, H. K. van Amstel, *MSX1* mutation is associated with orofacial clefting and tooth agenesis in humans. *Nat. Genet.* **24**, 342–343 (2000). doi: [10.1038/74155](https://doi.org/10.1038/74155); pmid: [10742093](https://pubmed.ncbi.nlm.nih.gov/10742093/)
 9. S. Kondo *et al.*, Mutations in *IRF6* cause Van der Woude and popliteal pterygium syndromes. *Nat. Genet.* **32**, 285–289 (2002). doi: [10.1038/ng985](https://doi.org/10.1038/ng985); pmid: [12219090](https://pubmed.ncbi.nlm.nih.gov/12219090/)
 10. S. Suzuki *et al.*, Mutations in *BMP4* are associated with subepithelial, microform, and overt cleft lip. *Am. J. Hum. Genet.* **84**, 406–411 (2009). doi: [10.1016/j.ajhg.2009.02.002](https://doi.org/10.1016/j.ajhg.2009.02.002); pmid: [19249007](https://pubmed.ncbi.nlm.nih.gov/19249007/)
 11. M. J. Dixon, Treacher Collins syndrome. *Hum. Mol. Genet.* **5**, 1391–1396 (1996). pmid: [8875242](https://pubmed.ncbi.nlm.nih.gov/8875242/)
 12. S. B. Ng *et al.*, Exome sequencing identifies *MLL2* mutations as a cause of Kabuki syndrome. *Nat. Genet.* **42**, 790–793 (2010). doi: [10.1038/ng.646](https://doi.org/10.1038/ng.646); pmid: [20711175](https://pubmed.ncbi.nlm.nih.gov/20711175/)
 13. S. B. Ng *et al.*, Exome sequencing identifies the cause of a Mendelian disorder. *Nat. Genet.* **42**, 30–35 (2010). doi: [10.1038/ng.499](https://doi.org/10.1038/ng.499); pmid: [19915526](https://pubmed.ncbi.nlm.nih.gov/19915526/)
 14. M. J. Dixon, M. L. Marazita, T. H. Beaty, J. C. Murray, Cleft lip and palate: Understanding genetic and environmental influences. *Nat. Rev. Genet.* **12**, 167–178 (2011). doi: [10.1038/nrg2933](https://doi.org/10.1038/nrg2933); pmid: [21331089](https://pubmed.ncbi.nlm.nih.gov/21331089/)
 15. S. Boehringer *et al.*, Genetic determination of human facial morphology: Links between cleft-lips and normal variation. *Eur. J. Hum. Genet.* **19**, 1192–1197 (2011). doi: [10.1038/ejhg.2011.110](https://doi.org/10.1038/ejhg.2011.110); pmid: [21694738](https://pubmed.ncbi.nlm.nih.gov/21694738/)
 16. F. Liu *et al.*, A genome-wide association study identifies five loci influencing facial morphology in Europeans. *PLoS Genet.* **8**, e1002932 (2012). doi: [10.1371/journal.pgen.1002932](https://doi.org/10.1371/journal.pgen.1002932); pmid: [23028347](https://pubmed.ncbi.nlm.nih.gov/23028347/)
 17. L. Paternoster *et al.*, Genome-wide association study of three-dimensional facial morphology identifies a variant in *PAX3* associated with nasion position. *Am. J. Hum. Genet.* **90**, 478–485 (2012). doi: [10.1016/j.ajhg.2011.12.021](https://doi.org/10.1016/j.ajhg.2011.12.021); pmid: [22341974](https://pubmed.ncbi.nlm.nih.gov/22341974/)
 18. D. L. Stern, Evolutionary developmental biology and the problem of variation. *Evolution* **54**, 1079–1091 (2000). pmid: [11005278](https://pubmed.ncbi.nlm.nih.gov/11005278/)
 19. Y. Shen *et al.*, A map of the cis-regulatory sequences in the mouse genome. *Nature* **488**, 116–120 (2012). doi: [10.1038/nature11243](https://doi.org/10.1038/nature11243); pmid: [22763441](https://pubmed.ncbi.nlm.nih.gov/22763441/)
 20. J. Zhu *et al.*, Genome-wide chromatin state transitions associated with developmental and environmental cues. *Cell* **152**, 642–654 (2013). doi: [10.1016/j.cell.2012.12.033](https://doi.org/10.1016/j.cell.2012.12.033); pmid: [23333102](https://pubmed.ncbi.nlm.nih.gov/23333102/)
 21. A. Visel *et al.*, ChIP-seq accurately predicts tissue-specific activity of enhancers. *Nature* **457**, 854–858 (2009). doi: [10.1038/nature07730](https://doi.org/10.1038/nature07730); pmid: [19212405](https://pubmed.ncbi.nlm.nih.gov/19212405/)
 22. N. M. Young, H. J. Chong, D. Hu, B. Hallgrímsson, R. S. Marcucio, Quantitative analyses link modulation of sonic hedgehog signaling to continuous variation in facial growth and shape. *Development* **137**, 3405–3409 (2010). doi: [10.1242/dev.052340](https://doi.org/10.1242/dev.052340); pmid: [20826528](https://pubmed.ncbi.nlm.nih.gov/20826528/)
 23. M. Simões-Costa, M. E. Bronner, Insights into neural crest development and evolution from genomic analysis. *Genome Res.* **23**, 1069–1080 (2013). doi: [10.1101/gr.157586.113](https://doi.org/10.1101/gr.157586.113); pmid: [23817048](https://pubmed.ncbi.nlm.nih.gov/23817048/)
 24. S. Bagheri-Fam *et al.*, Long-range upstream and downstream enhancers control distinct subsets of the complex spatiotemporal *Sox9* expression pattern. *Dev. Biol.* **291**, 382–397 (2006). doi: [10.1016/j.ydbio.2005.11.013](https://doi.org/10.1016/j.ydbio.2005.11.013); pmid: [16458883](https://pubmed.ncbi.nlm.nih.gov/16458883/)
 25. P. Betancur, M. Bronner-Fraser, T. Sauka-Spengler, Genomic code for *Sox10* activation reveals a key regulatory enhancer for cranial neural crest. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 3570–3575 (2010). doi: [10.1073/pnas.0906596107](https://doi.org/10.1073/pnas.0906596107); pmid: [20139305](https://pubmed.ncbi.nlm.nih.gov/20139305/)
 26. H. Yanagisawa, D. E. Clouthier, J. A. Richardson, J. Charité, E. N. Olson, Targeted deletion of a branchial arch-specific enhancer reveals a role of *dHAND* in craniofacial development. *Development* **130**, 1069–1078 (2003). doi: [10.1242/dev.00337](https://doi.org/10.1242/dev.00337); pmid: [12571099](https://pubmed.ncbi.nlm.nih.gov/12571099/)
 27. A. Rada-Iglesias *et al.*, Epigenomic annotation of enhancers predicts transcriptional regulators of human neural crest. *Cell Stem Cell* **11**, 633–648 (2012). doi: [10.1016/j.stem.2012.07.006](https://doi.org/10.1016/j.stem.2012.07.006); pmid: [22981823](https://pubmed.ncbi.nlm.nih.gov/22981823/)
 28. M. H. Kaufman, *The Atlas of Mouse Development* (Academic Press, London, 1992).
 29. W. Feng *et al.*, Spatial and temporal analysis of gene expression during growth and fusion of the mouse facial

- prominences. *PLOS ONE* **4**, e8066 (2009). doi: [10.1371/journal.pone.0008066](https://doi.org/10.1371/journal.pone.0008066); pmid: [20016822](https://pubmed.ncbi.nlm.nih.gov/20016822/)
30. Materials and methods are available as supplementary materials on Science Online.
 31. M. J. Blow *et al.*, ChIP-Seq identification of weakly conserved heart enhancers. *Nat. Genet.* **42**, 806–810 (2010). doi: [10.1038/ng.650](https://doi.org/10.1038/ng.650); pmid: [20729851](https://pubmed.ncbi.nlm.nih.gov/20729851/)
 32. A. Visel *et al.*, A high-resolution enhancer atlas of the developing telencephalon. *Cell* **152**, 895–908 (2013). doi: [10.1016/j.cell.2012.12.041](https://doi.org/10.1016/j.cell.2012.12.041); pmid: [23375746](https://pubmed.ncbi.nlm.nih.gov/23375746/)
 33. C. Y. McLean *et al.*, GREAT improves functional interpretation of cis-regulatory regions. *Nat. Biotechnol.* **28**, 495–501 (2010). doi: [10.1038/nbt.1630](https://doi.org/10.1038/nbt.1630); pmid: [20436461](https://pubmed.ncbi.nlm.nih.gov/20436461/)
 34. L. A. Pennacchio *et al.*, In vivo enhancer analysis of human conserved non-coding sequences. *Nature* **444**, 499–502 (2006). doi: [10.1038/nature05295](https://doi.org/10.1038/nature05295); pmid: [17086198](https://pubmed.ncbi.nlm.nih.gov/17086198/)
 35. J. Sharpe *et al.*, Optical projection tomography as a tool for 3D microscopy and gene expression studies. *Science* **296**, 541–545 (2002). doi: [10.1126/science.1068206](https://doi.org/10.1126/science.1068206); pmid: [11964482](https://pubmed.ncbi.nlm.nih.gov/11964482/)
 36. N. D. Heintzman *et al.*, Histone modifications at human enhancers reflect global cell-type-specific gene expression. *Nature* **459**, 108–112 (2009). doi: [10.1038/nature07829](https://doi.org/10.1038/nature07829); pmid: [19295514](https://pubmed.ncbi.nlm.nih.gov/19295514/)
 37. L. Z. Holland *et al.*, The amphioxus genome illuminates vertebrate origins and cephalochordate biology. *Genome Res.* **18**, 1100–1111 (2008). doi: [10.1101/gr.073676.107](https://doi.org/10.1101/gr.073676.107); pmid: [18562680](https://pubmed.ncbi.nlm.nih.gov/18562680/)
 38. D. May *et al.*, Large-scale discovery of enhancers from human heart tissue. *Nat. Genet.* **44**, 89–93 (2012). doi: [10.1038/ng.1006](https://doi.org/10.1038/ng.1006); pmid: [22138689](https://pubmed.ncbi.nlm.nih.gov/22138689/)
 39. A. Visel *et al.*, Ultraconservation identifies a small subset of extremely constrained developmental enhancers. *Nat. Genet.* **40**, 158–160 (2008). doi: [10.1038/ng.2007.55](https://doi.org/10.1038/ng.2007.55); pmid: [18176564](https://pubmed.ncbi.nlm.nih.gov/18176564/)
 40. H. Hochheiser *et al.*, The FaceBase Consortium: a comprehensive program to facilitate craniofacial research. *Dev. Biol.* **355**, 175–182 (2011). doi: [10.1016/j.ydbio.2011.02.033](https://doi.org/10.1016/j.ydbio.2011.02.033); pmid: [21458441](https://pubmed.ncbi.nlm.nih.gov/21458441/)
 41. S. Alappat, Z. Y. Zhang, Y. P. Chen, Msx homeobox gene family and craniofacial development. *Cell Res.* **13**, 429–442 (2003). doi: [10.1038/sj.cr.7290185](https://doi.org/10.1038/sj.cr.7290185); pmid: [14728799](https://pubmed.ncbi.nlm.nih.gov/14728799/)
 42. A. Visel, E. M. Rubin, L. A. Pennacchio, Genomic views of distant-acting enhancers. *Nature* **461**, 199–205 (2009). doi: [10.1038/nature08451](https://doi.org/10.1038/nature08451); pmid: [19741700](https://pubmed.ncbi.nlm.nih.gov/19741700/)
 43. S. Birnbaum *et al.*, Key susceptibility locus for nonsyndromic cleft lip with or without cleft palate on chromosome 8q24. *Nat. Genet.* **41**, 473–477 (2009). doi: [10.1038/ng.333](https://doi.org/10.1038/ng.333); pmid: [19270707](https://pubmed.ncbi.nlm.nih.gov/19270707/)
 44. E. Mangold *et al.*, Genome-wide linkage scan of nonsyndromic orofacial clefting in 91 families of central European origin. *Am. J. Med. Genet. A* **149A**, 2680–2694 (2009). doi: [10.1002/ajmg.a.33136](https://doi.org/10.1002/ajmg.a.33136); pmid: [19938073](https://pubmed.ncbi.nlm.nih.gov/19938073/)
 45. T. Nikopensius *et al.*, Replication of novel susceptibility locus for nonsyndromic cleft lip with or without cleft palate on chromosome 8q24 in Estonian and Lithuanian patients. *Am. J. Med. Genet. A* **149A**, 2551–2553 (2009). doi: [10.1002/ajmg.a.33024](https://doi.org/10.1002/ajmg.a.33024); pmid: [19839039](https://pubmed.ncbi.nlm.nih.gov/19839039/)
 46. T. H. Beaty *et al.*, A genome-wide association study of cleft lip with and without cleft palate identifies risk variants near MAFB and ABCA4. *Nat. Genet.* **42**, 525–529 (2010). doi: [10.1038/ng.580](https://doi.org/10.1038/ng.580); pmid: [20436469](https://pubmed.ncbi.nlm.nih.gov/20436469/)
 47. Q. Yuan, S. H. Blanton, J. T. Hecht, Association of ABCA4 and MAFB with non-syndromic cleft lip with or without cleft palate. *Am. J. Med. Genet. A* **155A**, 1469–1471 (2011). doi: [10.1002/ajmg.a.33940](https://doi.org/10.1002/ajmg.a.33940); pmid: [21567910](https://pubmed.ncbi.nlm.nih.gov/21567910/)
 48. E. J. Leslie *et al.*, Expression and mutation analyses implicate ARHGAP29 as the etiologic gene for the cleft lip with or without cleft palate locus identified by genome-wide association on chromosome 1p22. *Birth Defects Res. A Clin. Mol. Teratol.* **94**, 934–942 (2012). doi: [10.1002/bdra.23076](https://doi.org/10.1002/bdra.23076); pmid: [23008150](https://pubmed.ncbi.nlm.nih.gov/23008150/)
 49. F. Rahimov *et al.*, Disruption of an AP-2alpha binding site in an Irf6 enhancer is associated with cleft lip. *Nat. Genet.* **40**, 1341–1347 (2008). doi: [10.1038/ng.242](https://doi.org/10.1038/ng.242); pmid: [18836445](https://pubmed.ncbi.nlm.nih.gov/18836445/)
 50. K. F. Oram, E. A. Carver, T. Gridley, Slug expression during organogenesis in mice. *Anat. Rec. A Discov. Mol. Cell. Evol. Biol.* **271**, 189–191 (2003). doi: [10.1002/ar.a.10027](https://doi.org/10.1002/ar.a.10027); pmid: [12552634](https://pubmed.ncbi.nlm.nih.gov/12552634/)
 51. T. A. Mitsiadis, I. Angeli, C. James, U. Lendahl, P. T. Sharpe, Role of Islet1 in the patterning of murine dentition. *Development* **130**, 4451–4460 (2003). doi: [10.1242/dev.00631](https://doi.org/10.1242/dev.00631); pmid: [12900460](https://pubmed.ncbi.nlm.nih.gov/12900460/)
 52. D. E. Lieberman, B. Hallgrímsson, W. Liu, T. E. Parsons, H. A. Jamniczky, Spatial packing, cranial base angulation, and craniofacial shape variation in the mammalian skull: Testing a new model using mice. *J. Anat.* **212**, 720–735 (2008). doi: [10.1111/j.1469-7580.2008.00900.x](https://doi.org/10.1111/j.1469-7580.2008.00900.x); pmid: [18510502](https://pubmed.ncbi.nlm.nih.gov/18510502/)
 53. B. Hallgrímsson, D. E. Lieberman, W. Liu, A. F. Ford-Hutchinson, F. R. Jirik, Epigenetic interactions and the structure of phenotypic variation in the cranium. *Evol. Dev.* **9**, 76–91 (2007). doi: [10.1111/j.1525-142X.2006.00139.x](https://doi.org/10.1111/j.1525-142X.2006.00139.x); pmid: [17227368](https://pubmed.ncbi.nlm.nih.gov/17227368/)
 54. S. A. Murray, K. F. Oram, T. Gridley, Multiple functions of Snail family genes during palate development in mice. *Development* **134**, 1789–1797 (2007). doi: [10.1242/dev.02837](https://doi.org/10.1242/dev.02837); pmid: [17376812](https://pubmed.ncbi.nlm.nih.gov/17376812/)
 55. E. Mangold, K. U. Ludwig, M. M. Nöthen, Breakthroughs in the genetics of orofacial clefting. *Trends Mol. Med.* **17**, 725–733 (2011). doi: [10.1016/j.molmed.2011.07.007](https://doi.org/10.1016/j.molmed.2011.07.007); pmid: [21885341](https://pubmed.ncbi.nlm.nih.gov/21885341/)
 56. A. MacKenzie, L. Purdie, D. Davidson, M. Collinson, R. E. Hill, Two enhancer domains control early aspects of the complex expression pattern of Msx1. *Mech. Dev.* **62**, 29–40 (1997). doi: [10.1016/S0925-4773\(96\)00646-6](https://doi.org/10.1016/S0925-4773(96)00646-6); pmid: [9106164](https://pubmed.ncbi.nlm.nih.gov/9106164/)
 57. S. Yokoyama *et al.*, A systems approach reveals that the myogenesis genome network is regulated by the transcriptional repressor RP58. *Dev. Cell* **17**, 836–848 (2009). doi: [10.1016/j.devcel.2009.10.011](https://doi.org/10.1016/j.devcel.2009.10.011); pmid: [20059953](https://pubmed.ncbi.nlm.nih.gov/20059953/)
 58. B. Balczerski *et al.*, Distinct spatiotemporal roles of hedgehog signalling during chick and mouse cranial base and axial skeleton development. *Dev. Biol.* **371**, 203–214 (2012). doi: [10.1016/j.ydbio.2012.08.011](https://doi.org/10.1016/j.ydbio.2012.08.011); pmid: [23009899](https://pubmed.ncbi.nlm.nih.gov/23009899/)
 59. A. Vahtokari, T. Aberg, J. Jernvall, S. Keränen, I. Thesleff, The enamel knot as a signaling center in the developing mouse tooth. *Mech. Dev.* **54**, 39–43 (1996). doi: [10.1016/0925-4773\(95\)00459-9](https://doi.org/10.1016/0925-4773(95)00459-9); pmid: [8808404](https://pubmed.ncbi.nlm.nih.gov/8808404/)
 60. E. Koyama *et al.*, Polarizing activity, Sonic hedgehog, and tooth development in embryonic and postnatal mouse. *Dev. Dyn.* **206**, 59–72 (1996). doi: [10.1002/\(SICI\)1097-0177\(199605\)206:1<59::AID-AJA6>3.0.CO;2-#](https://doi.org/10.1002/(SICI)1097-0177(199605)206:1<59::AID-AJA6>3.0.CO;2-#); pmid: [9019247](https://pubmed.ncbi.nlm.nih.gov/9019247/)
 61. A. S. Tucker *et al.*, Conserved regulation of mesenchymal gene expression by Fgf-8 in face and limb development. *Development* **126**, 221–228 (1999). pmid: [9847236](https://pubmed.ncbi.nlm.nih.gov/9847236/)

Acknowledgments: The authors thank J. Harkes and M. Satyanarayanan for development of the OPT viewer; S. Shen and H. Hochheiser for integration of the OPT viewer and data sets into FaceBase; and J. Murray, M. Marazita, J. Manak, B. Schutte, and all FaceBase members for help in the selection of relevant craniofacial intervals and comments on results. A.V. and L.A.P. were supported by NIDCR FaceBase grant U01DE020060 and by National Human Genome Research Institute grants R01HG003988 and U54HG006997. C.A. was supported by a Swiss National Science Foundation advanced researcher fellowship. A.S.N. was supported by a F32 NIH/National Institute of General Medical Sciences National Research Service Award fellowship GM105202. B.H. was supported by NIH 1R01DE021708, NIH 1R01DE01963, NIH 1U01DE020054, and Natural Sciences and Engineering Research Council of Canada #238992-11 grants. D.R.F. and H.M. were supported by a UK Medical Research Council core program grant. B.R. was supported by the Ludwig Institute for Cancer Research and NIH grants U54HG006997 and R01HG003991. B.H. was supported by NIH 1R01DE01963. Research was conducted at the E. O. Lawrence Berkeley National Laboratory and performed under Department of Energy contract DE-AC02-05CH11231, University of California. ChIP-Seq data are available through GEO (accession no. GSE49413) and FaceBase.org. In vivo reporter data are available through the Vista Enhancer Browser (<http://enhancer.lbl.gov>) and FaceBase.org. OPT data, including raw images and interactive 3D viewing option, is available through <http://facebase.org>. All enhancer reporter vectors, as well as archived surplus LacZ-stained embryos for selected enhancers, are available from the authors. Craniofacial enhancer knockout lines are available through the Mutant Mouse Regional Resource Centers (Δ hs1431, MMRRC 03895; Δ hs746, MMRRC 03888; and Δ hs586, MMRRC 03894).

Supplementary Materials

www.sciencemag.org/content/342/6157/1241006/suppl/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S6
Tables S1 to S6
References (62–91)
Movies S1 to S20

24 May 2013; accepted 20 August 2013
10.1126/science.1241006

Voltage-Gated Sodium Channel in Grasshopper Mice Defends Against Bark Scorpion Toxin

Ashlee H. Rowe,^{1*†‡} Yucheng Xiao,^{2†} Matthew P. Rowe,^{3§} Theodore R. Cummins,² Harold H. Zakon^{1,4}

Painful venoms are used to deter predators. Pain itself, however, can signal damage and thus serves an important adaptive function. Evolution to reduce general pain responses, although valuable for preying on venomous species, is rare, likely because it comes with the risk of reduced response to tissue damage. Bark scorpions capitalize on the protective pain pathway of predators by inflicting intensely painful stings. However, grasshopper mice regularly attack and consume bark scorpions, grooming only briefly when stung. Bark scorpion venom induces pain in many mammals (house mice, rats, humans) by activating the voltage-gated Na⁺ channel Nav1.7, but has no effect on Nav1.8. Grasshopper mice Nav1.8 has amino acid variants that bind bark scorpion toxins and inhibit Na⁺ currents, blocking action potential propagation and inducing analgesia. Thus, grasshopper mice have solved the predator-pain problem by using a toxin bound to a nontarget channel to block transmission of the pain signals the venom itself is initiating.

Pain is adaptive in that it warns of tissue damage. Thus, pain sensitivity is essential for survival. Humans who lack pain sensitivity often suffer through regular injury (1). Many animals (e.g., jellyfish, ants, wasps, spiders, scorpions, vipers, stonefish, platypus) capitalize on the critical role of the sensory pain-pathway by producing venoms that induce extreme pain, likely as a mechanism for deterring predators (2). Immediate, intense pain may stun a predator, providing prey with the opportunity to escape. Longer term, the predator may avoid future encounters with known painful prey. While counter-selection on the predator might be expected, evolving sensory neurons that have a higher pain threshold may put animals at risk for injury and death from other environmental insults. Thus, the lack of examples documenting predators that are resistant to painful prey is not surprising.

Bark scorpions (*Centruroides* spp., Family Buthidae) are well known for inflicting intensely painful, potentially lethal stings (3–7). Grasshopper mice (*Onychomys* spp.), desert-dwelling

rodents that prey on scorpions, are resistant to *Centruroides*' lethal toxins (8). In this report, we examine whether the mice have also evolved resistance to the pain-inducing components in bark scorpion venom. Observations from a staged feeding study showed that southern grasshopper mice (*O. torridus*) sympatric with Arizona bark scorpions (*C. sculpturatus*, formerly known as *C. exilicauda*) responded to the scorpion's sting by grooming for only a few seconds before pressing their attack, voraciously killing and consuming the scorpion [(9), see also movie S1]. The mice's response was surprisingly brief given that

anecdotal reports describe *C. sculpturatus*' stings as producing an immediate burning sensation followed by prolonged throbbing pain that can last for hours. Our observations suggested that *O. torridus* have evolved reduced sensitivity to this painful venom. We here confirm that *O. torridus* are less sensitive than house mice (*Mus musculus*) to *C. sculpturatus*' pain-inducing toxins. We show that, in *O. torridus*, the channel (Nav1.8) responsible for transmitting pain signals to their central nervous system (CNS) has amino acid variants that bind venom peptides and inhibit channel current, paradoxically blocking pain signals instead of transmitting them.

Effects of Venom and Formalin on Sensory-Pain Behavior in Mice

We injected venom into the hind paws of *O. torridus* and *M. musculus*, using physiological saline as a control, and recorded the amount of time the mice spent licking their paws over a 15-min period. To select a venom dose that induces pain in the control species without lethal effects, we evaluated the response of *M. musculus* to three doses of venom having concentrations less than the median lethal dose (LD₅₀) (10) (fig. S1). *M. musculus* substantially increased their paw licking in response to venom as compared to saline, whereas *O. torridus* responded to venom by licking their paws only briefly (Fig. 1A). Indeed, *O. torridus* licked their paws less in response to venom than to saline.

To determine whether this response is specific to *C. sculpturatus* venom or represents a general insensitivity to painful stimuli, we injected formalin (11, 12) into the hind paws of *O. torridus* and *M. musculus* and recorded their paw licking for 15 min. Although *O. torridus* licked their paws less than *M. musculus* in response to formalin, the duration of licking was longer than their

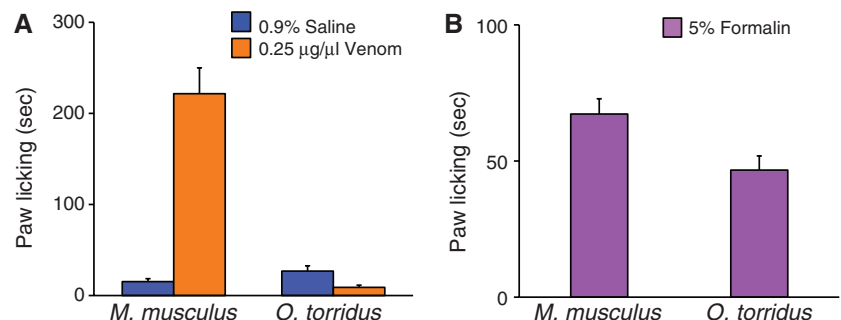


Fig. 1. Grasshopper mice (*O. torridus*) do not show pain responses to bark scorpion (*C. sculpturatus*) venom, but do to formalin. All data are shown as the mean + 1 SE. (A) *O. torridus* and *M. musculus* differed significantly in their response to venom. *M. musculus* increased their paw licking in response to venom as compared to saline (saline = 15.30 ± 3.22 s, venom = 221.63 ± 28.46 s, *n* = 8 mice). In contrast, *O. torridus* decreased their paw licking in response to venom as compared to saline (saline = 26.86 ± 5.78 s, *n* = 6 mice, venom = 8.98 ± 2.48 s, *n* = 8 mice). Two-way ANOVA species by treatment interaction, *F* (1,26) = 51.02, *P* < 0.0001. (B) Both *M. musculus* and *O. torridus* licked their paws in response to formalin (*M. musculus*: 67.26 ± 5.65 s; *O. torridus*: 46.67 ± 5.21 s; each species, *n* = 8 mice). However, *O. torridus* licked their paws significantly less than *M. musculus* in response to the treatment [one-way ANOVA, species by treatment interaction, *F* (1,14) = 7.18, *P* = 0.02].

¹Section of Neurobiology, The University of Texas at Austin, Austin, TX 78712, USA. ²Department of Pharmacology and Toxicology, Stark Neurosciences Research Institute, Indiana University School of Medicine, Indianapolis, IN 46202, USA. ³Department of Biological Sciences, Sam Houston State University, Huntsville, TX 77341, USA. ⁴Josephine Bay Paul Center for Comparative Molecular Biology and Evolution, Marine Biological Laboratory, Woods Hole, MA 02543, USA.

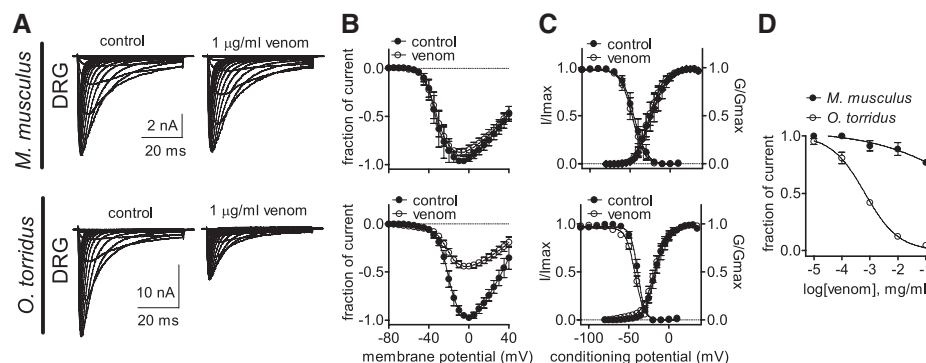
*Corresponding author. E-mail: roweashl@msu.edu

†These authors contributed equally to this work.

‡Present address: Neuroscience Program and Department of Zoology, Michigan State University, East Lansing, MI 48824, USA.

§Present address: Department of Zoology, Michigan State University, East Lansing, MI 48824, USA.

Fig. 2. *C. sculpturatus* venom inhibits *O. torridus*, but not *M. musculus* TTX-R Na^+ current. (A) Current traces and (B) the current-voltage relation demonstrate that venom inhibits *O. torridus* TTX-R Na^+ currents recorded from native channels expressed in small-diameter DRG neurons but has no effect on *M. musculus* TTX-R currents. Currents were elicited by 50-ms depolarizing steps to voltages ranging from -80 to 40 mV in 5 -mV increments. All currents induced before (control) and after the application of venom were normalized to the maximum amplitude of control peak current. (C) Venom did not significantly affect the voltage dependence of channel activation or steady-state inactivation in either species. The voltage dependence of steady-state inactivation was estimated with a standard double-pulse protocol in which Na^+ currents were induced by a 20 -ms depolarizing potential of 0 mV following a 500 -ms prepulse at voltages ranging from -110 to 10 mV. Currents were plotted as a fraction of the maximum peak current. Data points were fitted with the Boltzmann equation [$M. musculus$ $V_{1/2}$ for activation: control = -45.0 ± 1.4 mV, venom = -47.0 ± 1.2 mV; $V_{1/2}$ for steady-state inactivation: control = -24.3 ± 1.5 mV, venom = -26.0 ± 1.1 mV), (*O. torridus* $V_{1/2}$ for activation: control = -38.7 ± 0.4 mV, venom = -42.2 ± 0.5 mV; $V_{1/2}$ for steady-state inactivation: control = -17.1 ± 0.7 mV, venom = -18.1 ± 0.6 mV]. (D) Venom inhibited *O. torridus*' DRG-expressed TTX-R Na^+ current



in a concentration-dependent manner ($\text{IC}_{50} = 0.7 \mu\text{g/ml}$), whereas high concentrations of venom had little effect on *M. musculus* TTX-R current ($\text{IC}_{50} = 5.7 \text{ mg/ml}$). The inhibitory response was assessed with a single pulse protocol in which current was elicited every 5 s by a 50 -ms depolarizing potential of 0 mV. Data points were fit with the Hill logistic equation: $Y = \text{bottom} + (\text{top} - \text{bottom}) / (1 + 10^{((\log \text{EC}_{50} - X) \times \text{Hill slope})})$, where EC_{50} is median effective concentration. For (A) to (D), dissociated DRG cells were pretreated with 500 nM TTX to block TTX-S Na^+ channels and held at -80 mV ($n = 4$ or 5 cells obtained from two different animals of each species). For (B) to (D), data are shown as the mean ± 1 SE.

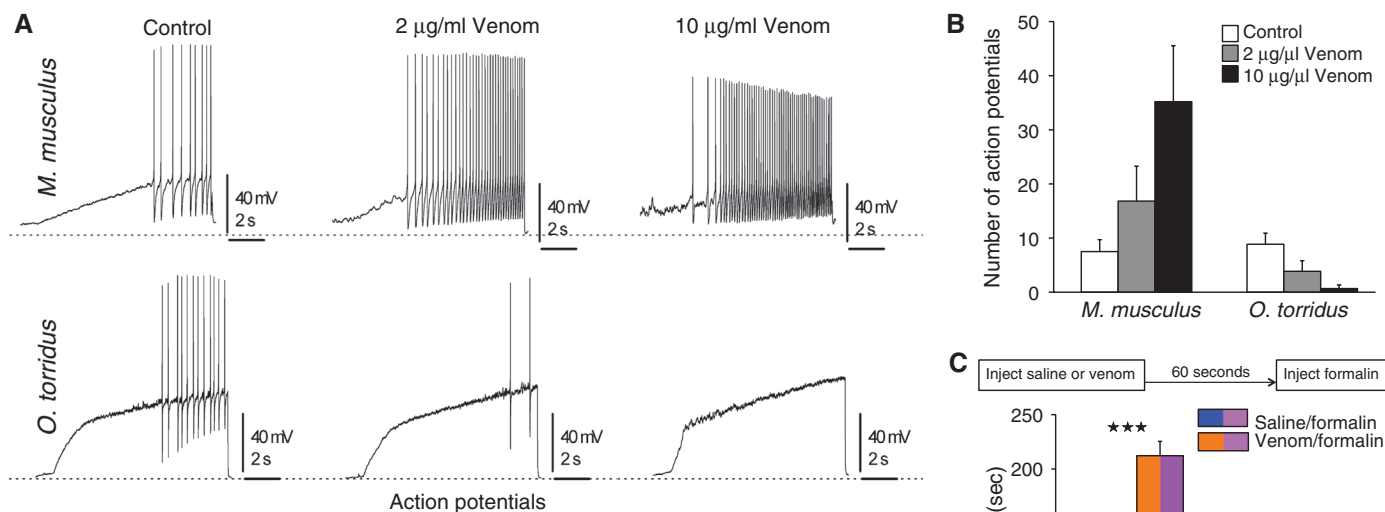


Fig. 3. *C. sculpturatus* venom decreases membrane excitability and action potential firing in *O. torridus*' small-diameter DRG neurons, reducing their sensitivity to formalin. (A) Ramp currents were injected into DRG neurons to produce small, slow depolarization, similar to that evoked by pain-inducing stimuli. Whereas venom increased membrane excitability and action potential (AP) firing in *M. musculus* DRG neurons, it decreased excitability and blocked AP firing in *O. torridus* DRG neurons. (B) *O. torridus* and *M. musculus* differed significantly in their response to increasing doses of venom. Venom increased the number of APs generated by *M. musculus* DRG neurons (control = 7.5 ± 2.2 APs, $2 \mu\text{g/ml}$ venom = 16.83 ± 6.45 APs, $10 \mu\text{g/ml}$ venom = 35.2 ± 10.33 APs, $n = 6$ cells from 2 different animals), but decreased the number of APs generated by *O. torridus* DRG neurons (control = 8.85 ± 2.05 APs, $2 \mu\text{g/ml}$ venom = 3.85 ± 1.96 APs, $10 \mu\text{g/ml}$ venom = 0.66 ± 0.66 APs, $n = 7$ cells from 3 different animals). Data are shown as the mean number of APs (± 1 SE), multivariate ANOVA with repeated measures, species by treatment interaction, $F(2, 8) = 7.52$, $P = 0.01$. (C) Preinjecting *M. musculus* with $0.25 \mu\text{g}/\mu\text{l}$ venom significantly increased their paw licking in response to an injection of 0.1% formalin as compared to preinjecting with 0.9% saline (saline/formalin = 36.35 ± 4.84 s, venom/formalin = 212.25 ± 13.16 s, $n = 16$ mice, paired t test, $***P < 0.0001$). In contrast, preinjecting *O. torridus* with $0.25 \mu\text{g}/\mu\text{l}$ venom significantly decreased their paw licking in response to an injection of 0.5% formalin as compared to preinjecting with 0.9% saline (saline/formalin = 26.14 ± 4.19 s, venom/formalin = 12.47 ± 3.03 s, $n = 16$ mice, paired t test, $**P = 0.01$). Data are shown as the mean duration (± 1 SE) of paw licking recorded for 20 min after the second injection in each treatment. Two-way ANOVA with repeated measures, species by treatment interaction, $F(1, 30) = 145.42$, $P < 0.0001$. For each species, one group of 16 mice was used for both treatments (i.e., each mouse served as its own control). Each mouse received one set of injections to a right or left hind paw and 1 week later received the second set of injections in the alternate paw (order of injections into the right or left hind paw was counterbalanced).

(B) *O. torridus* and *M. musculus* differed significantly in their response to increasing doses of venom. Venom increased the number of APs generated by *M. musculus* DRG neurons (control = 7.5 ± 2.2 APs, $2 \mu\text{g/ml}$ venom = 16.83 ± 6.45 APs, $10 \mu\text{g/ml}$ venom = 35.2 ± 10.33 APs, $n = 6$ cells from 2 different animals), but decreased the number of APs generated by *O. torridus* DRG neurons (control = 8.85 ± 2.05 APs, $2 \mu\text{g/ml}$ venom = 3.85 ± 1.96 APs, $10 \mu\text{g/ml}$ venom = 0.66 ± 0.66 APs, $n = 7$ cells from 3 different animals). Data are shown as the mean number of APs (± 1 SE), multivariate ANOVA with repeated measures, species by treatment interaction, $F(2, 8) = 7.52$, $P = 0.01$. (C) Preinjecting *M. musculus* with $0.25 \mu\text{g}/\mu\text{l}$ venom significantly increased their paw licking in response to an injection of 0.1% formalin as compared to preinjecting with 0.9% saline (saline/formalin = 36.35 ± 4.84 s, venom/formalin = 212.25 ± 13.16 s, $n = 16$ mice, paired t test, $***P < 0.0001$). In contrast, preinjecting *O. torridus* with $0.25 \mu\text{g}/\mu\text{l}$ venom significantly decreased their paw licking in response to an injection of 0.5% formalin as compared to preinjecting with 0.9% saline (saline/formalin = 26.14 ± 4.19 s, venom/formalin = 12.47 ± 3.03 s, $n = 16$ mice, paired t test, $**P = 0.01$). Data are shown as the mean duration (± 1 SE) of paw licking recorded for 20 min after the second injection in each treatment. Two-way ANOVA with repeated measures, species by treatment interaction, $F(1, 30) = 145.42$, $P < 0.0001$. For each species, one group of 16 mice was used for both treatments (i.e., each mouse served as its own control). Each mouse received one set of injections to a right or left hind paw and 1 week later received the second set of injections in the alternate paw (order of injections into the right or left hind paw was counterbalanced).

response to saline, demonstrating that they are sensitive to alternative painful stimuli (Fig. 1B). Although *M. musculus* found the venom to be substantially more irritating than formalin, a post hoc comparison showed that *O. torridus* found the injections of formalin to be more irritating than saline, and the saline to be more irritating than the venom (fig. S2).

Effects of Venom on Dorsal Root Ganglion–Expressed TTX-R Na^+ Current

In mammals, acute pain (nociception) is transmitted to the CNS mainly by two voltage-gated sodium (Na^+) channels, tetrodotoxin-sensitive (TTX-S) Nav1.7 and tetrodotoxin-resistant (TTX-R) Nav1.8, that are expressed in small-diameter dorsal root ganglion (DRG) neurons (nociceptors) (13). Nociceptors also express Nav1.9, a second TTX-R Na^+ current involved in diabetic neuropathy and inflammatory pain (13–17). Venoms from Old and New World Buthidae scorpions initiate acute pain in sensitive mammals (e.g., house mice, rats, humans) by activating Nav1.7, but have no effect on Nav1.8 (18–21). We used TTX to block Nav1.7 and confirmed that *C. sculpturatus* venom does not affect DRG-expressed TTX-R Na^+ current in *M. musculus* (this TTX-R current is primarily, if not exclusively,

due to Nav1.8 as Nav1.9 loses activity rapidly in dissociated neurons) (Fig. 2, A and B). However, venom inhibited TTX-R Na^+ currents recorded from dissociated DRG neurons in *O. torridus* (Fig. 2, A and B). Venom did not significantly affect the voltage dependence of channel activation or steady-state inactivation in either species (Fig. 2C). Increasing venom concentrations had no effect on *M. musculus* but decreased *O. torridus* TTX-R Na^+ currents in a dose-dependent manner (Fig. 2D).

Effects of Venom on Nociceptor Excitability and Action Potential Generation

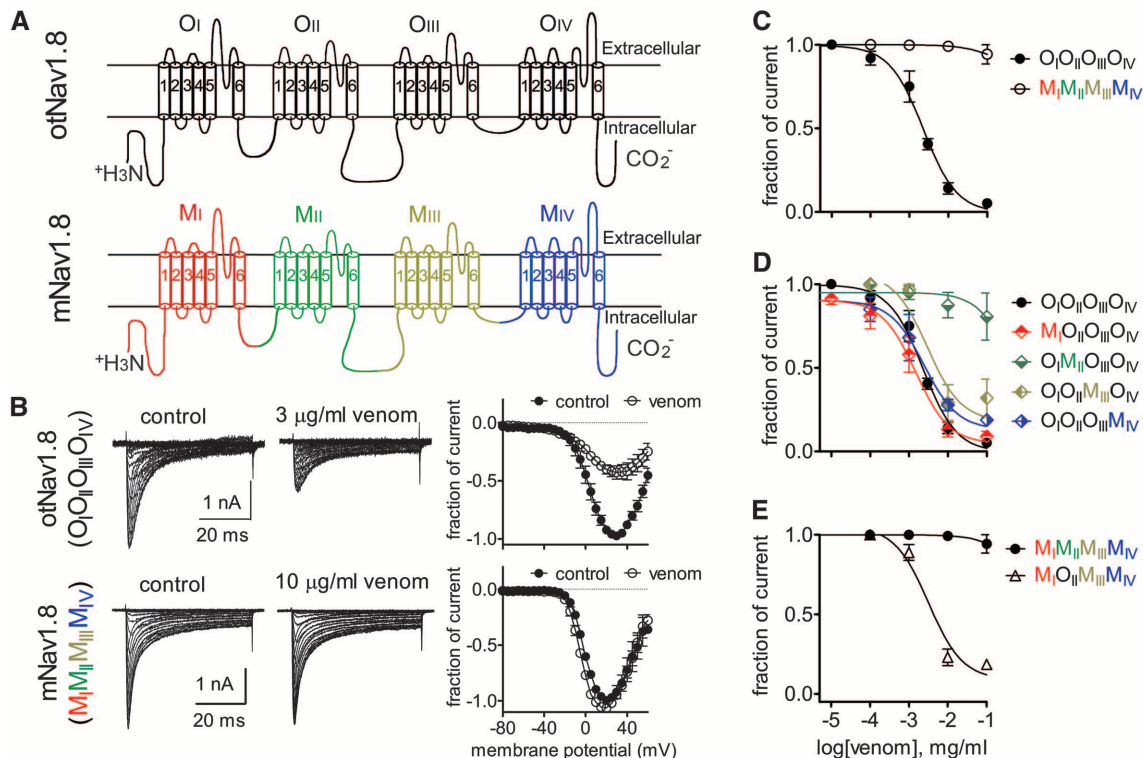
Empirical and modeling studies show that whereas Nav1.7 initiates action potentials in nociceptors, Nav1.8 Na^+ currents are necessary for their sustained firing and propagation (13, 22–24). Thus, we predicted that venom-induced inhibition of Nav1.8 Na^+ currents would decrease membrane excitability and block action potential propagation in *O. torridus* nociceptors. To test this, we examined the effects of venom on action potential firing in small-diameter DRG neurons. We recorded baseline firing by injecting ramp currents into dissociated DRG neurons to mimic the small, slow depolarization of membranes elicited by noxious stimuli. *O. torridus* and *M. musculus* differed

in DRG neuron current threshold, with *O. torridus* neurons requiring significantly more current than *M. musculus* neurons to reach baseline excitability (fig. S3). After DRG neurons in both species reached an equivalent baseline level of firing, we measured the effect of venom on nociceptor excitability. Venom significantly increased membrane excitability and action potential firing in *M. musculus* neurons, but had the opposite effect on *O. torridus* neurons, in which venom significantly decreased membrane excitability and blocked action potential firing (Fig. 3, A and B). Instead of inducing pain in *O. torridus*, *C. sculpturatus* venom paradoxically blocks pain signaling to the CNS. By blocking signals to the CNS, it follows that venom should also reduce *O. torridus*' response to subsequent painful stimuli; that is, venom may induce analgesia in *O. torridus*.

Effects of Venom on Response to Alternative Painful Stimuli

To determine whether venom induces analgesia in *O. torridus*, we examined paw-licking responses to 5% formalin injected into a hind paw after an injection of venom. Formalin concentrations >1% induce a biphasic response in rodents; an immediate, brief response mediated, in part, by the nociceptor-expressed transient receptor potential

Fig. 4. Domain II is the major contributor to the sensitivity of *O. torridus* Nav1.8 to *C. sculpturatus* venom. (A) Schematic diagram of sodium channel α subunit Nav1.8 from *O. torridus* (otNav1.8) and *M. musculus* (mNav1.8). O_I to O_{IV} and M_I to M_{IV} represent four different domains of otNav1.8 and mNav1.8, respectively. (B) *C. sculpturatus* venom inhibited otNav1.8 TTX-resistant Na^+ current, but not mNav1.8, expressed by ND7/23 cells. Cells were pretreated with 500 nM TTX to block TTX-sensitive (i.e., Nav1.7) currents and held at –80 mV. Na^+ currents were induced by 50-ms depolarizing steps to various potentials ranging from –80 to +60 mV in 5-mV increments. All currents elicited before (control) and after venom treatment



were normalized to the maximum amplitude of control peak current. (C) Concentration-response inhibitory curves for *C. sculpturatus* venom on wild-type otNav1.8 (O_IO_{II}O_{III}O_{IV}, filled circles) and mNav1.8 (M_IM_{II}M_{III}M_{IV}, open circles). The IC₅₀ for otNav1.8 was 2.2 $\mu\text{g/ml}$. (D) Effects of *C. sculpturatus* venom on four otNav1.8/mNav1.8 chimeras. Each of the four domains in otNav1.8 was replaced by the corresponding domain from mNav1.8. Venom inhibited Na^+ current expressed by chimeras where domains I, III, and IV were

exchanged (IC₅₀ values: M_IO_{II}O_{III}O_{IV} = 1.5 $\mu\text{g/ml}$; O_IO_{II}M_{III}O_{IV} = 3.0 $\mu\text{g/ml}$; O_IO_{II}O_{III}M_{IV} = 2.4 $\mu\text{g/ml}$). Venom had no effect on the domain II chimera (IC₅₀ value: O_IM_{II}O_{III}O_{IV} > 524.6 $\mu\text{g/ml}$). (E) The reverse replacement of otNav1.8-DII significantly increased the sensitivity of mNav1.8 to *C. sculpturatus* venom (IC₅₀ value: M_IO_{II}M_{III}M_{IV} = 3.2 $\mu\text{g/ml}$). Each data point is shown as the mean $\pm$ SE. Data for (C) to (E) were obtained from 3 to 7 separate cells expressing each channel construct.

cation channel (TRPA1), and a delayed, persistent response mediated by inflammatory agents and changes to the CNS (11, 12, 25, 26). To minimize responses due to tissue inflammation, we measured paw licking during the 5-min period immediately after formalin injections. A two-way analysis of variance (ANOVA) revealed a significant species-by-treatment interaction. *M. musculus* licked their paws more in response to formalin after pretreatment with venom as compared to formalin without venom pretreatment (fig. S4). However, *O. torridus* decreased their paw licking in response to formalin when pretreated with venom (fig. S4). We replicated this study using lower concentrations of formalin (<1%) to elicit nociceptor-mediated paw licking and further minimize inflammation-induced licking. We accomplished this by selecting doses of formalin that produced paw licking within the first 10 min after formalin injections, but that produced little or no licking after 10 min (fig. S5). The results of the replicated study confirmed that pretreating with venom before formalin injections significantly increased paw licking in *M. musculus* but significantly decreased paw licking in *O. torridus* (Fig. 3C).

Nav1.8 Molecular Structure

Voltage-gated Na⁺ channels are membrane-spanning proteins constructed of four homologous domains (DI to DIV), with six transmembrane segments (S1 to S6) composing each domain (27–29) (fig. S6A). Positively charged residues

in S4 of each domain serve as sensors that detect changes in membrane voltage to regulate channel gating. The reentrant loop between S5 and S6 in each domain join to form the channel pore. Venoms of buthid scorpions such as *C. sculpturatus* consist of multiple, low-molecular weight peptides that bind either to the amino acids in the extracellular loops connecting the transmembrane segments or to the residues lining the pore of the channel (10, 30–34). Because *C. sculpturatus* venom inhibits TTX-R Na⁺ current in *O. torridus* Nav1.8 but has no effect on *M. musculus*, we predicted that the structure of Nav1.8 would differ between the two species. To identify structural differences, we cloned and sequenced the gene (*Scn10a*) that encodes Nav1.8 from *O. torridus* and compared it to orthologous sequence from *M. musculus*. We identified multiple amino acid variants in *O. torridus* Nav1.8 (fig. S6, A and B). Many of these variants were distributed throughout the extracellular loops and pore region of the channel where they would be accessible to venom peptides.

Nav1.8 Chimeras

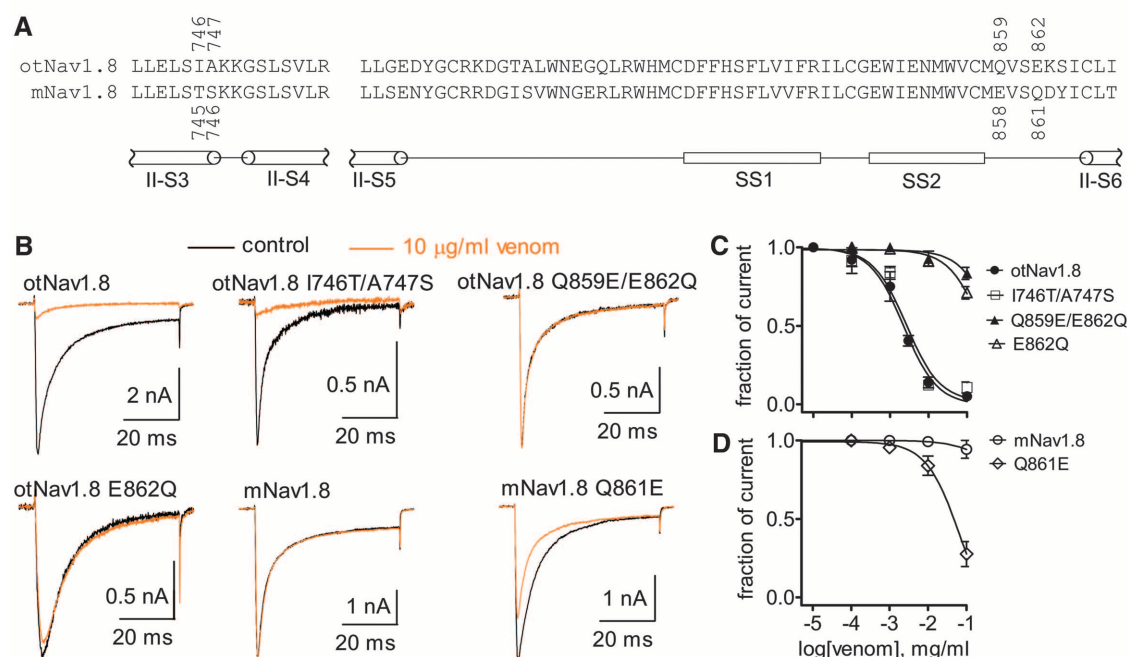
To identify the region of the channel critical for venom-induced inhibition of Na⁺ current, we made constructs of Nav1.8 for *O. torridus* (otNav1.8) and *M. musculus* (mNav1.8) by inserting *Scn10a* from each species into expression vectors (Fig. 4A). We confirmed that *C. sculpturatus* venom had no effect on mNav1.8 Na⁺ current but dose depen-

dently blocked otNav1.8 (Fig. 4, B and C). Using otNav1.8 as the framework, we made four chimeras in which each domain of otNav1.8 was replaced with the corresponding domain from mNav1.8. We tested venom on the chimeras and found that replacing domain I in otNav1.8 did not affect venom activity, and replacing domains III and IV only slightly reduced venom activity (Fig. 4D). However, replacing domain II in otNav1.8 abolished the effects of the venom on the channel (Fig. 4D). Moreover, reversing the chimera by using mNav1.8 as the framework and replacing domain II with the corresponding domain from otNav1.8, imparted venom sensitivity to mNav1.8 (Fig. 4E). *C. sculpturatus* venom dose dependently inhibited mNav1.8 Na⁺ current after exchanging domain II with otNav1.8. Thus, domain II is the major contributor to venom sensitivity in otNav1.8.

Site-Directed Mutagenesis

To identify the amino acid(s) in domain II that impart venom sensitivity to otNav1.8, we used site-directed mutagenesis to replace amino acid variants in otNav1.8 domain II with residues corresponding to the same positions in mNav1.8. Initially we focused on two amino acid variants expressed in *O. torridus* domain II S3–S4 because scorpion peptides known to target domain II in other Na⁺ channels bind to amino acids in this loop (31). We replaced two hydrophobic amino acids (I⁷⁴⁶ and

Fig. 5. An acidic residue (E862) in domain II near the pore region (S52–S6 linker) plays a critical role in the sensitivity of otNav1.8 to *C. sculpturatus* venom. (A) The amino acid sequence representing domain II S3–S4 and S5–S6 linkers from otNav1.8 is aligned with the corresponding sequence from mNav1.8. The position of each amino acid residue of interest is designated with a number. (B) Effects of *C. sculpturatus* venom on wild-type and mutant Nav1.8 channels expressed by ND7/23 cells. Cells were pretreated with 500 nM TTX and held at –80 mV. Representative current traces before (control, black) and after 10 μg/ml venom treatment (orange) were elicited by a 50-ms depolarizing potential of +20 mV. (C) Concentration-response inhibitory curves show the effect of *C. sculpturatus* venom on wild-type and mutant otNav1.8 channels. *C. sculpturatus* venom IC₅₀ values were estimated to be >251.4 μg/ml on the single-mutant E862Q and >531.5 μg/ml on the double-mutant Q859E/E862Q, respectively. (D) The reverse mutation Q861E in mNav1.8 increased the sensitivity of the channel to *C. sculpturatus*



venom. The IC₅₀ value was 66.9 μg/ml for the mutant Q861E. Each data point is shown as the mean ± SE. Data for (C) and (D) were obtained from 3 to 7 separate cells expressing each channel construct. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

A⁷⁴⁷) in the otNav1.8 domain II S3-S4 with hydrophilic residues (T⁷⁴⁶ and S⁷⁴⁷) that correspond to the same positions in mNav1.8 (Fig. 5A). However, *C. sculpturatus* venom dose dependently blocked Na⁺ current in both the wild-type construct (otNav1.8) and the mutant (otNav1.8 I746T/A747S) (Fig. 5, B and C), demonstrating that these variants do not contribute to venom sensitivity in *O. torridus*.

We then focused on two amino acid variants expressed in *O. torridus* domain II SS2-S6 linker, a structure that is adjacent to the channel pore. In *O. torridus* SS2-S6 loop, the positions of a hydrophilic (Q⁸⁵⁹) and an acidic (E⁸⁶²) residue are reversed as compared to corresponding residues in *M. musculus* (Fig. 5A). We made a construct in which we changed the hydrophilic (Q⁸⁵⁹) and acidic (E⁸⁶²) residues in OtNav1.8 to acidic (E⁸⁵⁹) and hydrophilic (Q⁸⁶²) residues, respectively. *C. sculpturatus* venom had no effect on the double mutant (otNav1.8 Q859E/E862Q), suggesting not only that an acidic residue is important for the activity of the venom, but also that the position of the acidic residue is critical for venom sensitivity (Fig. 5, B and C). To confirm this, we replaced the acidic residue (E⁸⁶²) with the hydrophilic (Q⁸⁶²) residue and found that *C. sculpturatus*

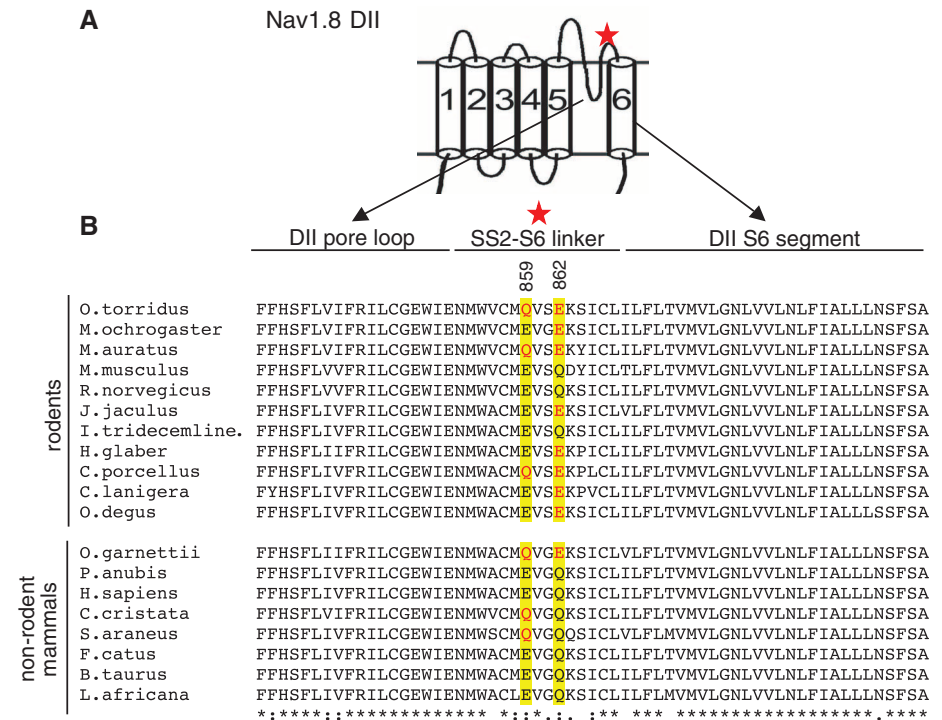
venom had almost no effect on Na⁺ current expressed by the single mutant (otNav1.8 E862Q) (Fig. 5, B and C). These results demonstrate that glutamic acid (E⁸⁶²) expressed adjacent to the pore at position 862 is critical for imparting venom sensitivity to otNav1.8. Further, changing the hydrophilic residue (Q⁸⁶¹) to glutamic acid (E⁸⁶¹) imparted venom sensitivity to the *M. musculus* construct as *C. sculpturatus* venom inhibited Na⁺ current in the single mutant (mNav1.8 Q861E) (Fig. 5, B and D). However, whereas replacement of domain II in mNav1.8 with domain II from otNav1.8 inhibited most of the Na⁺ current in the mutant with a median inhibitory concentration (IC₅₀) similar to that of the otNav1.8 wild type, the Q⁸⁶¹E replacement in mNav1.8 did not completely block Na⁺ current. These results suggest that glutamic acid (E⁸⁶²) is necessary for TTX-R Na⁺ current inhibition in *O. torridus* Nav1.8, but that additional amino acid variants in domain II may contribute to venom sensitivity.

Amino Acid Variants Critical for Venom-Pain Insensitivity Occur Frequently in Rodent and Nonrodent Mammals

To determine whether the amino acids critical for venom-pain insensitivity are unique to *O. torridus*,

we compared orthologous sequence from 18 species, including rodents and nonrodents from 15 mammalian families (Fig. 6). The sequence alignment revealed that all 18 species express either glutamic acid (E) or glutamine (Q) at positions 859 and 862. The amino acid variants (Q⁸⁵⁹ and E⁸⁶²) observed in *O. torridus* occurred frequently in both closely and distantly related rodents as well as nonrodent mammals. The glutamic acid (E⁸⁶²) that is critical for blocking Na⁺ current in *O. torridus* Nav1.8 was expressed in 7 of the 10 rodent species and one primate. Two of these rodents (*Mesocricetus auratus*, *Cavia porcellus*), as well as the primate (*Otolemur garnettii*), also expressed the glutamine (Q⁸⁵⁹) residue. Conservation of positions 859 and 862 for expression of either glutamic acid or glutamine across a diverse group of mammals suggests that these amino acids play a critical role in the structure and function of the Nav1.8 domain II SS2-S6 linker. However, positions 859 and 862 are not conserved by the particular order in which the amino acids are expressed. All four possible permutations for the two amino acids are expressed across the species shown in the alignment. Thus, although E⁸⁶² most likely evolved in a mammalian ancestor under selection pressures

Fig. 6. *O. torridus* Nav1.8 DII SS2-S6 linker amino acid variants critical for venom insensitivity are expressed frequently in rodent and nonrodent mammals. (A) Schematic diagram of domain II from a typical voltage-gated Na⁺ channel α subunit designating the DII SS2-S6 linker (red star) where *O. torridus* expresses amino acid variants critical for venom-pain insensitivity. **(B)** An alignment of *O. torridus* Nav1.8 DII with orthologous sequence from rodent and nonrodent mammals shows that the extracellular SS2-S6 linker is more variable than flanking intracellular regions (DII pore loop and DII S6 segment). However, all species shown express either glutamic acid (E) or glutamine (Q) at positions 859 and 862 (yellow background), suggesting that these sites are conserved. Ten species, including rodents and nonrodent mammals, express either one or both of the amino acid variants (site 859, Q shown in red; site 862, E shown in red) observed in *O. torridus* that are critical for venom insensitivity. *M. auratus*, *C. porcellus*, and *O. garnettii* express Q at site 859 and E at site 862. *M. ochrogaster*, *J. jaculus*, *H. glaber*, *C. lanigera*, and *O. degus* express E at site 862. *C. cristata* and *S. araneus* express Q at site 859. Sequences were downloaded from the NCBI and aligned with the Clustal format (MAFFT L-INS-1, v6.850b). Sequences were arranged in descending order according to taxonomic family and relatedness to *O. torridus*. Identical amino acids are shown as (*), highly conserved amino acid variants as (:), similar amino acid variants as (.), and dissimilar amino acid variants or gaps as a blank (). Rodents [Cricetidae: *O. torridus* (southern grasshopper mouse), *Microtus ochrogaster* (prairie vole, XM_005348098), *Mesocricetus auratus* (golden hamster, XM_005348098); Muridae: *Mus musculus* (house mouse, NM_001205321), *Rattus norvegicus* (Norway rat, NM_017247); Dipodidae: *Jaculus jaculus* (lesser Egyptian jerboa, XM_004662101); Sciuridae: *Ictidomys tridecemlineatus* (thirteen-lined ground squirrel, XM_005317385); Bathyergidae: *Heterocephalus glaber* (naked mole-rat, JF_912495); Caviidae:



Cavia porcellus (guinea pig, XM_003464141); Chinchillidae: *Chinchilla lanigera* (long-tailed chinchilla, XM_005386574); Octodontidae: *Octodon degus* (degu, XM_004642215)]. Nonrodent mammals [Galagidae: *Otolemur garnettii* (small-eared galago, XM_003794468); Cercopithecidae: *Papio anubis* (olive baboon, XM_003894661); Hominidae: *Homo sapiens* (human, NM_006514); Talpidae: *Condylura cristata* (star-nosed mole, XM_004676631); Soricidae: *Sorex araneus* (European shrew, XM_004614563); Felidae: *Felis catus* (domestic cat, XM_003992249); Bovidae: *Bos taurus* (cattle, XM_002696916); Elephantidae: *Loxodonta africana* (African elephant, XM_003415747)].

that were not associated with scorpion venom, *O. torridus* can use this negatively charged amino acid to reduce their sensitivity to venom-induced pain.

Discussion

Pain sensitivity is critical for survival. Thus, many animals use painful venom to deter predators. Because voltage-gated Na^+ channels play a major role in transmitting sensory-pain signals to the CNS, they are often the targets of pain-inducing venoms. *C. sculpturatus* venom induces pain by activating the channel (Nav1.7) responsible for initiating pain signaling in nociceptors. Although pain-inducing venom could impose strong selection on the receiver, counteradaptation may be constrained by the risks associated with reduced pain sensitivity. Given the important role of Na^+ channels in pain signaling, counteradaptation may also be constrained by the conservation of Na^+ channel structure and function. However, *O. torridus* can sustain multiple stings during predatory attacks on *C. sculpturatus*. In *O. torridus*, the channel (Nav1.8) responsible for transmitting pain signals to their CNS has amino acid variants that bind venom peptides and inhibit channel current, blocking pain signals instead of transmitting them. This mechanism represents a unique evolutionary strategy as many examples of resistance to deadly toxins involve structural modifications to the target channel or receptor that interfere with toxin binding [e.g., resistance to cobra toxin (35, 36), resistance to tetrodotoxin (37–40)]. In *O. torridus*, however, a channel (Nav1.8) that is not the target of the venom has amino acid variants that facilitate venom binding. Moreover, scorpion peptides that target Na^+ channels typically activate the channel or block inactivation, prolonging channel activity and increasing neuron excitability (31, 32). In contrast, scorpion peptides inhibit *O. torridus* Nav1.8 Na^+ current and decrease neuron excitability, blocking neuronal signaling and inducing analgesia.

A fascinating parallel to the grasshopper mouse–bark scorpion case involves African naked mole rats that are insensitive to acid-induced pain (41, 42). Naked mole rats live in subterranean colonies where they are exposed to high concentrations of carbon dioxide (CO_2). Increased CO_2 environments induce pain by activating proton-gated acid sensors expressed in nociceptors. Instead of modifications to the proton-gated acid sensors, naked mole rats evolved amino acid variants in Nav1.7, the channel responsible for initiating pain signals in nociceptors. In naked mole rats, protons bind to the pore region of Nav1.7 and block Na^+ current, inhibiting action potential initiation and preventing pain signaling. Thus, in naked mole rats, a nontarget Na^+ channel expressed in the sensory pain pathway evolved structural variations that facilitate proton binding, ultimately blocking the pain signals that the protons are initiating.

The pain-reducing mechanisms observed in southern grasshopper mice and African naked

mole rats are exciting because they demonstrate that although Na^+ channels are structurally conserved, variation exists among species. Moreover, slight variations in Na^+ channel structure can produce substantial physiological effects. Reversing the positions of a hydrophilic and an acidic amino acid in Nav1.8 is critical for imparting venom-pain insensitivity to *O. torridus*, and converting a positively charged amino acid motif to a negatively charged motif in Nav1.7 renders naked mole rats insensitive to acid-induced pain. Because the amino acid variants in *O. torridus* Nav1.8 and naked mole rat Nav1.7 are each directed against the source of the pain (venom peptides and protons, respectively), *O. torridus* can exploit a biochemically protected food resource and naked mole rats can live in subterranean colonies while circumventing the constraints associated with evolving generally desensitized nociceptors. Future studies should examine whether there has been counterselection on *C. sculpturatus*, perhaps resulting in peptides modified to overcome the mice's resistance to this scorpion's painful sting. Given that venom toxins and their ion-channel targets are both products of gene families, *C. sculpturatus* and *O. torridus* provide an excellent model for investigating coevolution and arms races at the molecular and biochemical levels.

C. sculpturatus and *O. torridus* also provide a unique model for analgesia studies. Nav1.7 is considered a target for analgesic development because of its role in human pain disorders (13). However, our results demonstrate the key role Nav1.8 plays in pain signaling and its potential to serve as an analgesic target. Moreover, given that few toxins have been identified that bind Nav1.8, and none that bind selectively (43), the molecular and biochemical interactions between venom peptides and Nav1.8 could serve as the basis for designing highly selective, nonaddictive analgesics.

References and Notes

1. J. J. Cox *et al.*, *Nature* **444**, 894–898 (2006).
2. J. O. Schmidt, in *Insect Defenses: Adaptive Mechanisms and Strategies of Prey and Predators*, D. L. Evans, Ed. (State University of New York Press, Albany, NY, 1990), pp. 383–417.
3. S. C. Curry, M. V. Vance, P. J. Ryan, D. B. Kunkel, W. T. Northey, *J. Toxicol. Clin. Toxicol.* **21**, 417–449 (1983).
4. V. Fet, G. Lowe, in *Catalog of the Scorpions of the World (1758–1998)*, V. Fet, W. D. Sissom, G. Lowe, M. E. Braunwalder, Eds. (New York Entomological Society, New York, 2000), vol. 1, pp. 54–286.
5. F. LoVecchio, C. McBride, *J. Toxicol. Clin. Toxicol.* **41**, 937–940 (2003).
6. L. V. Boyer *et al.*, *N. Engl. J. Med.* **360**, 2090–2098 (2009).
7. A. B. Skolnik, M. B. Ewald, *Pediatr. Emerg. Care* **29**, 98–103, quiz 104–105 (2013).
8. A. H. Rowe, M. P. Rowe, *Toxicon* **52**, 597–605 (2008).
9. A. H. Rowe, M. P. Rowe, *Anim. Behav.* **71**, 725–734 (2006).
10. J. M. Simard, H. Meves, D. D. Watt, in *Natural Toxins: Toxicology, Chemistry and Safety*, R. F. Keeler, N. B. Mandava, A. T. Tu, Eds. (Alaken, Fort Collins, CO, 1992), vol. 1, pp. 236–263.
11. A. Tjølset, O. G. Berge, S. Hunskaar, J. H. Rosland, K. Hole, *Pain* **51**, 5–17 (1992).
12. K. J. Sufka, G. S. Watson, R. E. Nothdurft, J. S. Mogil, *Eur. J. Pain* **2**, 351–358 (1998).
13. S. D. Dib-Hajj, T. R. Cummins, J. A. Black, S. G. Waxman, *Annu. Rev. Neurosci.* **33**, 325–347 (2010).
14. T. R. Cummins *et al.*, *J. Neurosci.* **19**, RC43 (1999).
15. S. Dib-Hajj, J. A. Black, T. R. Cummins, S. G. Waxman, *Trends Neurosci.* **25**, 253–259 (2002).
16. S. Lollignier *et al.*, *PLOS ONE* **6**, e23083 (2011).
17. S. Leo, R. D'Hooge, T. Meert, *Behav. Brain Res.* **208**, 149–157 (2010).
18. C. Y. Saab, T. R. Cummins, S. D. Dib-Hajj, S. G. Waxman, *Neurosci. Lett.* **331**, 79–82 (2002).
19. C. Maertens *et al.*, *Mol. Pharmacol.* **70**, 405–414 (2006).
20. E. R. Moraes, E. Kalapothakis, L. A. Naves, C. Kushmerick, *Neurotox. Res.* **19**, 102–114 (2011).
21. A. H. Rowe *et al.*, *PLOS ONE* **6**, e23520 (2011).
22. M. Renganathan, T. R. Cummins, S. G. Waxman, *J. Neurophysiol.* **86**, 629–640 (2001).
23. N. T. Blair, B. P. Bean, *J. Neurosci.* **22**, 10277–10290 (2002).
24. J.-S. Choi, S. G. Waxman, *J. Neurophysiol.* **106**, 3173–3184 (2011).
25. C. R. McNamara *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 13525–13530 (2007).
26. S. D. Shields, D. J. Cavanaugh, H. Lee, D. J. Anderson, A. I. Basbaum, *Pain* **151**, 422–429 (2010).
27. W. A. Catterall, *Physiol. Rev.* **72** (suppl.), S15–S48 (1992).
28. W. A. Catterall, *Neuron* **26**, 13–25 (2000).
29. W. A. Catterall, A. L. Goldin, S. G. Waxman, International Union of Pharmacology, *Pharmacol. Rev.* **57**, 397–409 (2005).
30. J. C. Rogers, Y. Qu, T. N. Tanada, T. Scheuer, W. A. Catterall, *J. Biol. Chem.* **271**, 15950–15962 (1996).
31. S. Cestèle *et al.*, *Neuron* **21**, 919–931 (1998).
32. R. C. Rodríguez de la Vega, L. D. Possani, *Toxicon* **46**, 831–844 (2005).
33. J. Z. Zhang *et al.*, *J. Biol. Chem.* **287**, 30719–30728 (2012).
34. M. Gurevitz, *Toxicon* **60**, 502–511 (2012).
35. H. J. Kreienkamp, S. M. Sine, R. K. Maeda, P. Taylor, *J. Biol. Chem.* **269**, 8108–8114 (1994).
36. Z. Takacs, K. C. Wilhelmsen, S. Sorota, *Mol. Biol. Evol.* **18**, 1800–1809 (2001).
37. Y. Kaneko, G. Matsumoto, Y. Hanyu, *Biochem. Biophys. Res. Commun.* **240**, 651–656 (1997).
38. M. Yotsu-Yamashita *et al.*, *Biochem. Biophys. Res. Commun.* **267**, 403–412 (2000).
39. S. Geffeney, E. D. Brodie Jr., P. C. Ruben, E. D. Brodie 3rd, *Science* **297**, 1336–1339 (2002).
40. B. Venkatesh *et al.*, *Curr. Biol.* **15**, 2069–2072 (2005).
41. T. J. Park *et al.*, *PLOS Biol.* **6**, e13 (2008).
42. E. S. Smith *et al.*, *Science* **334**, 1557–1560 (2011).
43. J. Gilchrist, F. Bosmans, *Toxins* **4**, 620–632 (2012).

Acknowledgments: We thank M. Heitlinger, the Santa Rita Experimental Range (Univ. of Arizona), and the Arizona Game and Fish Department for assistance with animal collections. We also thank Y. Lu and M. Wu for technical assistance with Nav1.8 sequencing and construct assembly. This work was supported, in part or in whole, by NSF IOS Award 1122115 (to A.H.R., M.P.R., and H.H.Z.), Department of the Army grants W911NF-06-1-0213 and W911NF-09-1-0355 from the Army Research Office Life Sciences Division (to A.H.R. and H.H.Z.), NIH grant NS 053422 from the National Institute of Neurological Disorders and Stroke (to T.R.C. and Y.X.), and a Faculty Research Grant from the Office of Research and Sponsored Projects, Sam Houston State University (to M.P.R.). The Department of Biological Sciences at Sam Houston State University provided logistical support for fieldwork. We declare no conflict of interest. The data are included in the main article and in the supplementary materials. The gene (*Scn10a*) encoding *O. torridus* Nav1.8 has been deposited in NCBI's GenBank gene database. Information about the GenBank accession numbers can be found in the supplementary materials.

Supplementary Materials

www.sciencemag.org/content/342/6157/441/suppl/DC1
Materials and Methods
Figs. S1 to S6
Movie S1

12 February 2013; accepted 27 September 2013
10.1126/science.1236451

Mucus Enhances Gut Homeostasis and Oral Tolerance by Delivering Immunoregulatory Signals

Meimei Shan,¹ Maurizio Gentile,² John R. Yeiser,¹ A. Cooper Walland,¹ Victor U. Bornstein,¹ Kang Chen,^{1,3} Bing He,¹ Linda Cassis,² Anna Bigas,⁴ Montserrat Cols,¹ Laura Comerma,^{2,5} Bihui Huang,⁶ J. Magarian Blander,^{1,7} Huabao Xiong,¹ Lloyd Mayer,¹ Cecilia Berin,⁸ Leonard H. Augenlicht,⁹ Anna Velcich,⁹ Andrea Cerutti^{1,2,10,11*}

A dense mucus layer in the large intestine prevents inflammation by shielding the underlying epithelium from luminal bacteria and food antigens. This mucus barrier is organized around the hyperglycosylated mucin MUC2. Here we show that the small intestine has a porous mucus layer, which permitted the uptake of MUC2 by antigen-sampling dendritic cells (DCs). Glycans associated with MUC2 imprinted DCs with anti-inflammatory properties by assembling a galectin-3–Dectin-1–FcγRIIB receptor complex that activated β-catenin. This transcription factor interfered with DC expression of inflammatory but not tolerogenic cytokines by inhibiting gene transcription through nuclear factor κB. MUC2 induced additional conditioning signals in intestinal epithelial cells. Thus, mucus does not merely form a nonspecific physical barrier, but also constrains the immunogenicity of gut antigens by delivering tolerogenic signals.

Mechanisms whereby the gut mucosa tolerates commensal bacteria and food antigens without developing inflamma-

tion remain elusive. Though traditionally viewed as a nonspecific barrier between the host and the environment, mucus also regulates gut homeostasis.

The building block of gut mucus is MUC2, a gel-forming mucin secreted by goblet cells (GCs) (1). In the large intestine (LI), MUC2 prevents inflammation by generating an outer nonattached mucus layer inhabited by the microbiota and an inner mucus layer adherent to intestinal epithelial cells (IECs) and impervious to bacteria (1). The structure and function of mucus in the small intestine (SI) are less well understood.

¹Department of Medicine, Immunology Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA. ²Program for Inflammatory and Cardiovascular Disorders, Institut Hospital del Mar d'Investigacions Mèdiques (IMIM), Barcelona, Spain. ³Department of Obstetrics and Gynecology, Wayne State University, Detroit, MI, USA. ⁴Program for Cancer Research, IMIM, Barcelona, Spain. ⁵Department of Pathology, Hospital del Mar, Barcelona, Spain. ⁶Department of Pharmacology, Yale University, New Haven, CT, USA. ⁷Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA. ⁸Department of Pediatrics, Immunology Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA. ⁹Department of Medicine, Albert Einstein College of Medicine, New York, NY, USA. ¹⁰Mucosal Immunology Studies Team (MIST), National Institutes of Health, Bethesda, MD, USA. ¹¹Catalan Institute for Research and Advanced Studies (ICREA), Barcelona, Spain.

*Corresponding author. E-mail: andrea.cerutti@mssm.edu or acerutti@imim.es

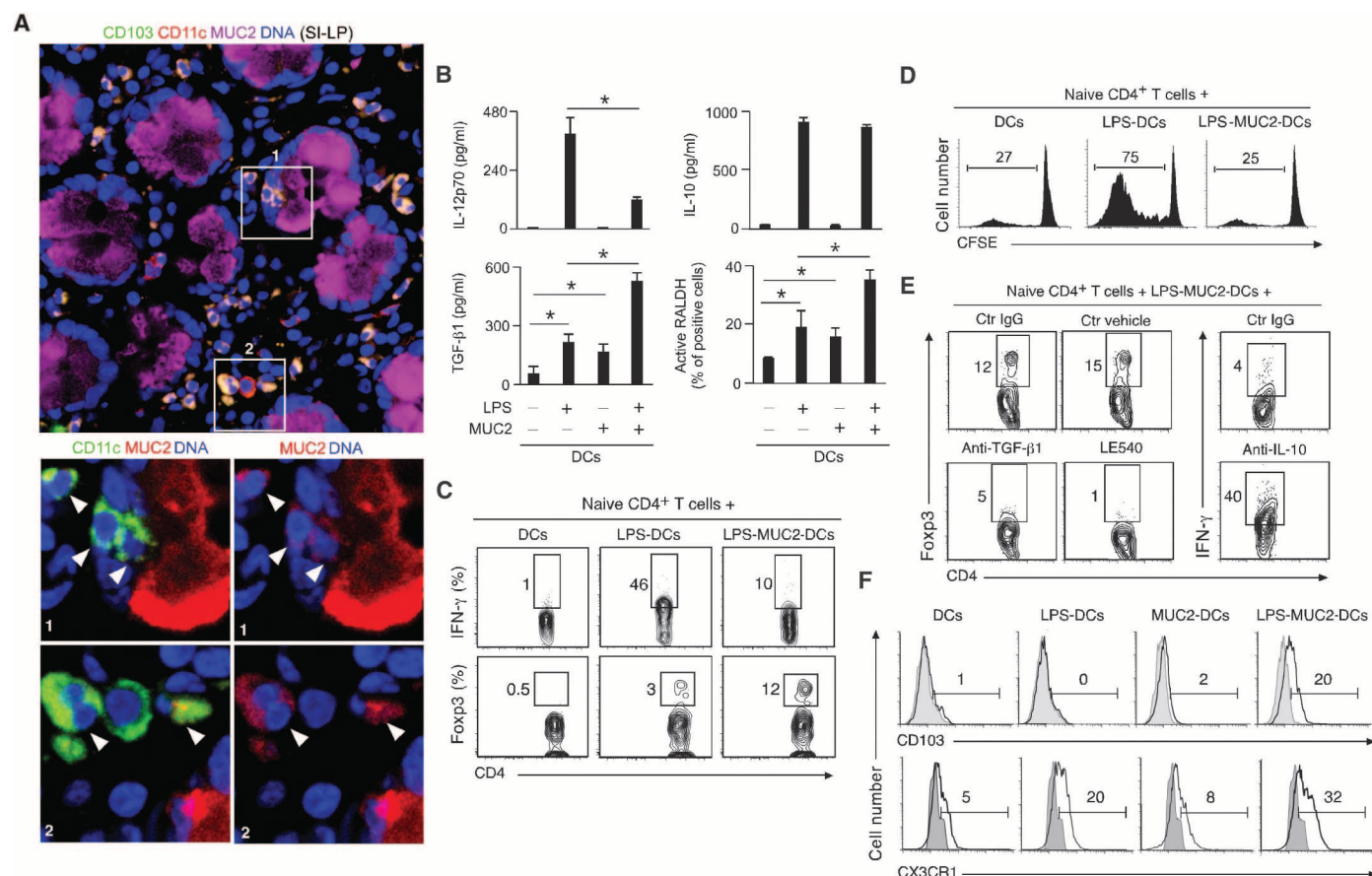


Fig. 1. MUC2 imprints DCs with tolerogenic properties. (A) Confocal microscopy of human SI-LP stained for CD11c, MUC2, CD103 and DNA-capturing 4'-6-diamidino-2-phenylindole (DAPI). Boxes and arrowheads: MUC2+CD103+ DCs. Original magnification, x63. (B) Enzyme-linked immunosorbent assay (ELISA) of IL-12p70, IL-10, and TGF-β1 and flow cytometry (FC) of active RALDH in human DCs cultured for 2 days with or without LPS and/or MUC2. (C) FC of

Naive CD4+ T cells cultured for 4 days with allogeneic DCs stimulated with or without LPS and/or MUC2 for 2 or 5 days in the absence or presence of control (ctr) IgG Ab, ctr vehicle, neutralizing Abs to TGF-β1 or IL-10, or LE540. (F) FC of CD103 and CX3CR1 on DCs cultured for 2 days with or without LPS and/or MUC2. Data summarize three experiments (error bars, SD; unpaired *t* test, **P* < 0.05) or show one of four experiments with similar results.

The SI harbors bacteria that promote homeostasis by inducing Foxp3⁺ T regulatory (T_{reg}) cells and B cell production of immunoglobulin A (IgA) antibodies (Abs) in Peyer's patches (PPs) and mesenteric lymph nodes (MLNs) (2, 3). These responses involve sampling of bacteria by CD11c⁺ dendritic cells (DCs) (4), including macrophage-like CD103⁺CD11b⁺CX3CR1⁺ DCs and myeloid CD103⁺CD11b⁺CX3CR1⁻ DCs (2, 5, 6). In the lamina propria (LP), myeloid DCs sample soluble antigens from GC-associated passages and cooperate with lymphoid CD103⁺CD11b⁺CX3CR1⁻ DCs to generate T_{reg} cells (7–9). Such antigen-sampling activities require a porous mucus barrier, raising questions as to how MUC2 prevents inflammation in the SI.

MUC2 Mitigates Inflammatory Responses in DCs

We first analyzed the structure of gut mucus in wild-type C57BL/6 mice. Whereas LI mucus formed a dense bilayered barrier that segregated bacteria from IECs, SI mucus was less organized and thus permitted the adhesion of bacteria to IECs (fig. S1, A to C). Consistent with the pres-

ence of some MUC2-coated bacteria on IECs and inside DCs (fig. S2, A to C), CX3CR1⁺ DCs from both PPs and SI-LP captured carboxyfluorescein succinimidyl ester (CFSE)-labeled MUC2 bound to fluorescent bacteria (fig. S3, A to C). In agreement with recent studies (6), some CD103⁺ DCs too captured MUC2-coated bacteria (fig. S3, A to D). Similarly, human monocyte-derived DCs internalized MUC2-bound bacteria across IECs sealed by occludin-containing tight junctions (fig. S4, A to D). Moreover, MUC2 was detected in human SI-LP CD103⁺ DCs proximal to GCs (Fig. 1A and movies S1 and S2).

In the presence of MUC2, human DCs exposed to bacteria or lipopolysaccharide (LPS) across IECs or directly incubated with LPS secreted less interleukin-12 (IL-12) (Fig. 1B and fig. S4E), a cytokine that induces proinflammatory interferon- γ (IFN- γ)-producing CD4⁺ T helper 1 (T_H1) cells (2). This effect was comparably induced by human, murine, or porcine MUC2 and was not due to elevated endotoxin, impaired DC uptake of bacteria, or increased DC apoptosis (figs. S4F and S5, A to D). Unlike native MUC2,

deglycosylated MUC2, a MUC2 peptide, or the mucin-interacting protein trefoil factor 3 did not inhibit LPS-induced IL-12 secretion (fig. S5E). MUC2 also impaired IL-12 as well as IL-6, IL-8, and tumor necrosis factor (TNF) transcription in response to bacterial Toll-like receptor (TLR) ligands such as LPS and flagellin or cytokines such as TNF (Fig. 1B and fig. S6, A to E). MUC2 elicited similar anti-inflammatory effects in monocyte-derived and myeloid CD11c⁺ DCs (Fig. 1B and fig. S6, A to E), which may include precursors of mucosal DCs (2, 8, 10). Thus, uptake of MUC2 causes carbohydrate-dependent attenuation of proinflammatory cytokine production by DCs.

MUC2 Delivers Tolerogenic Signals to DCs

Next, we established whether MUC2 induces IL-10, an anti-inflammatory cytokine that inhibits IL-12 and IFN- γ (2). Besides sustaining or augmenting IL-10 transcription and secretion in monocyte-derived DCs exposed to LPS or flagellin, MUC2 enhanced IL-10 secretion in LPS-activated myeloid CD11c⁺ DCs (Fig. 1B and fig. S6, C to E). MUC2 alone or combined with LPS

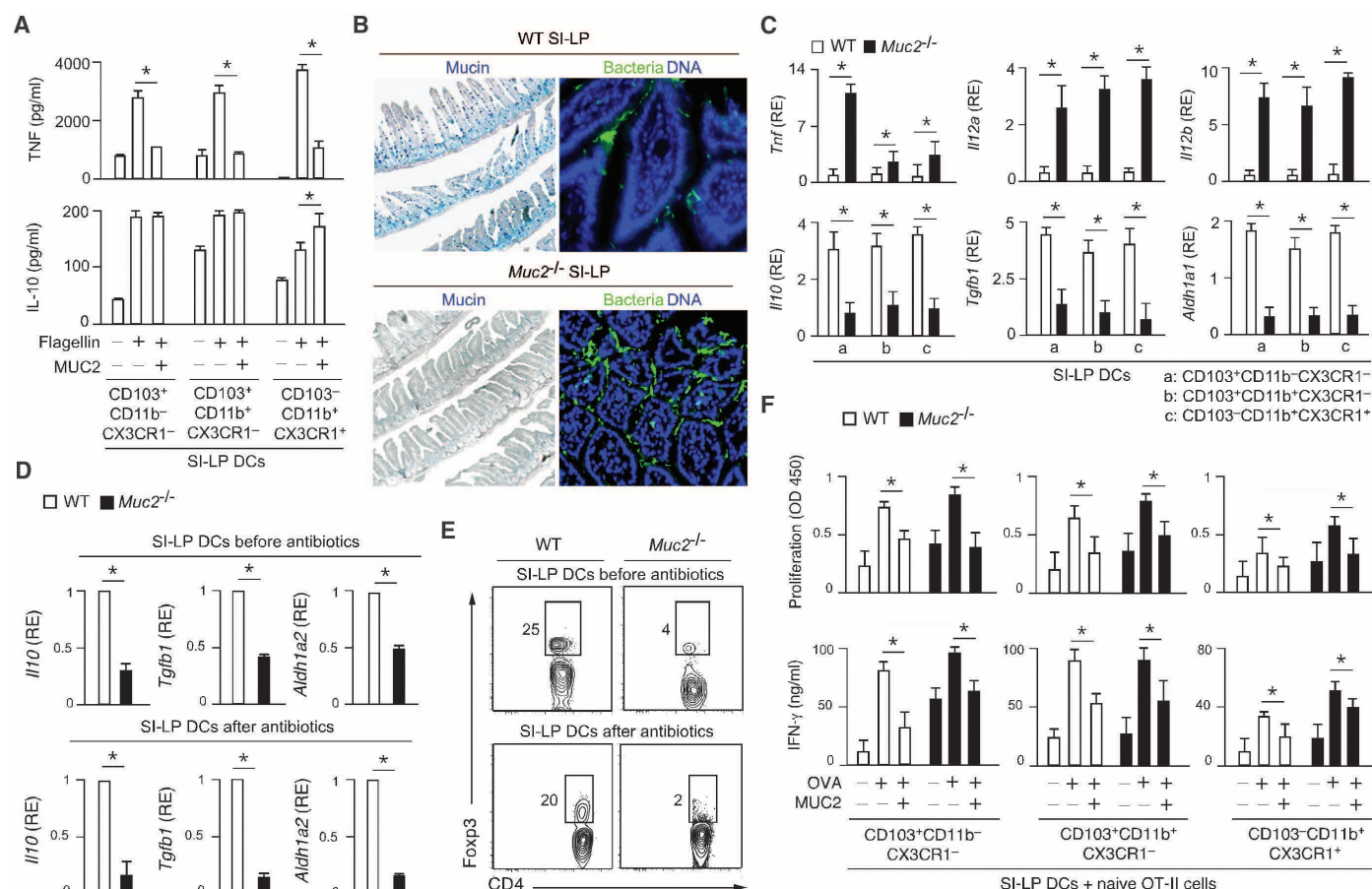


Fig. 2. MUC2 delivers anti-inflammatory signals to gut DCs. (A) ELISA of TNF and IL-10 from mouse SI-LP DCs cultured for 2 days with or without flagellin and/or MUC2. (B) Light microscopy of Alcian blue–stained mucin and fluorescence in situ hybridization (FISH) of bacterial 16S ribosomal RNA in DAPI-stained SI-LP from wild-type (WT) and *Muc2*^{-/-} mice. Original magnification, $\times 10$. (C to E) Quantitative real-time polymerase chain reaction (qRT-PCR) of mRNAs for TNF, IL-12p35 (*Il12a*), IL-12p40 (*Il12b*), IL-10, RALDH1 (*Aldh1a1*), and TGF- β 1 in SI-LP

DCs and FC of Foxp3 and CD4 on SI-LP T cells from WT and *Muc2*^{-/-} mice before and after oral antibiotics. RE, relative expression compared to *Gapdh* encoding glyceraldehyde 3-phosphate dehydrogenase. (F) ELISA of proliferation-induced bromodeoxyuridine (BrdU) and IFN- γ from OT-II cells activated for 5 days by OVA-pulsed SI-LP DCs from WT and *Muc2*^{-/-} mice with or without MUC2. Data summarize two experiments with ≥ 3 mice per group (error bars, SD; unpaired Student's *t* test, **P* < 0.05) or show one of four experiments with similar results.

also increased DC transcription and secretion of transforming growth factor- β 1 (TGF- β 1), a SMAD-signaling cytokine (Fig. 1B and figs. S6E and S7A) that helps the induction of T_{reg} cells by tolerogenic CD103⁺ DCs (2, 7, 11). Moreover, MUC2 augmented DC transcription and activation of retinaldehyde dehydrogenase (RALDH or ALDH1), a 4-diethylaminobenzaldehyde (DEAB)-sensitive enzyme (Fig. 1B and figs. S6E and S7B) with A1-3 isoforms that help CD103⁺ DCs to induce T_{reg} cells by converting dietary vitamin A into retinoic acid (RA) (2, 7, 11).

Accordingly, human DCs exposed to LPS in the presence of MUC2 decreased CD4⁺ T cell proliferation and IFN- γ production, but increased Foxp3 expression (Fig. 1, C and D, and fig. S8A). These effects were reversed by neutralizing Abs to IL-10 or TGF- β 1 and by the RA antagonist LE540 (Fig. 1E). Despite up-regulating the antigen-presenting molecule human leukocyte antigen-DR (HLA-DR), MUC2 down-regulated the T cell costimulatory molecules CD80 and CD86 and the maturation molecule CD83 on LPS-activated DCs (fig. S8B). Consistent with its ability to autonomously induce TGF- β 1 and RA, MUC2 induced

regulatory DCs even in the absence of LPS priming (fig. S9, A and B). In addition to up-regulating CD103 and CX3CR1 on LPS-activated DCs (Fig. 1F), MUC2 stimulated CD103 expression and T_{reg} cell-inducing signals in DCs undergoing trans-epithelial sampling of bacteria (fig. S10, A to C).

In the presence of MUC2, LPS-primed mouse bone marrow-derived DCs produced less IL-12 but more IL-10 (fig. S11A). When pulsed with the soluble protein ovalbumin (OVA) in the presence of MUC2, these DCs triggered less IFN- γ production by OVA-specific transgenic OT-II CD4⁺ T cells, which concurrently generated more T_{reg} cells (fig. S11, B and C). MUC2 also attenuated flagellin-induced TNF but sustained or augmented IL-10 production in SI-LP CD103⁺ and CX3CR1⁺ DCs, respectively (Fig. 2A). Thus, MUC2 elicits tolerogenic IL-10, TGF- β 1, and RA signals that somewhat vary in distinct subsets of DCs.

MUC2 Enhances Gut Homeostasis

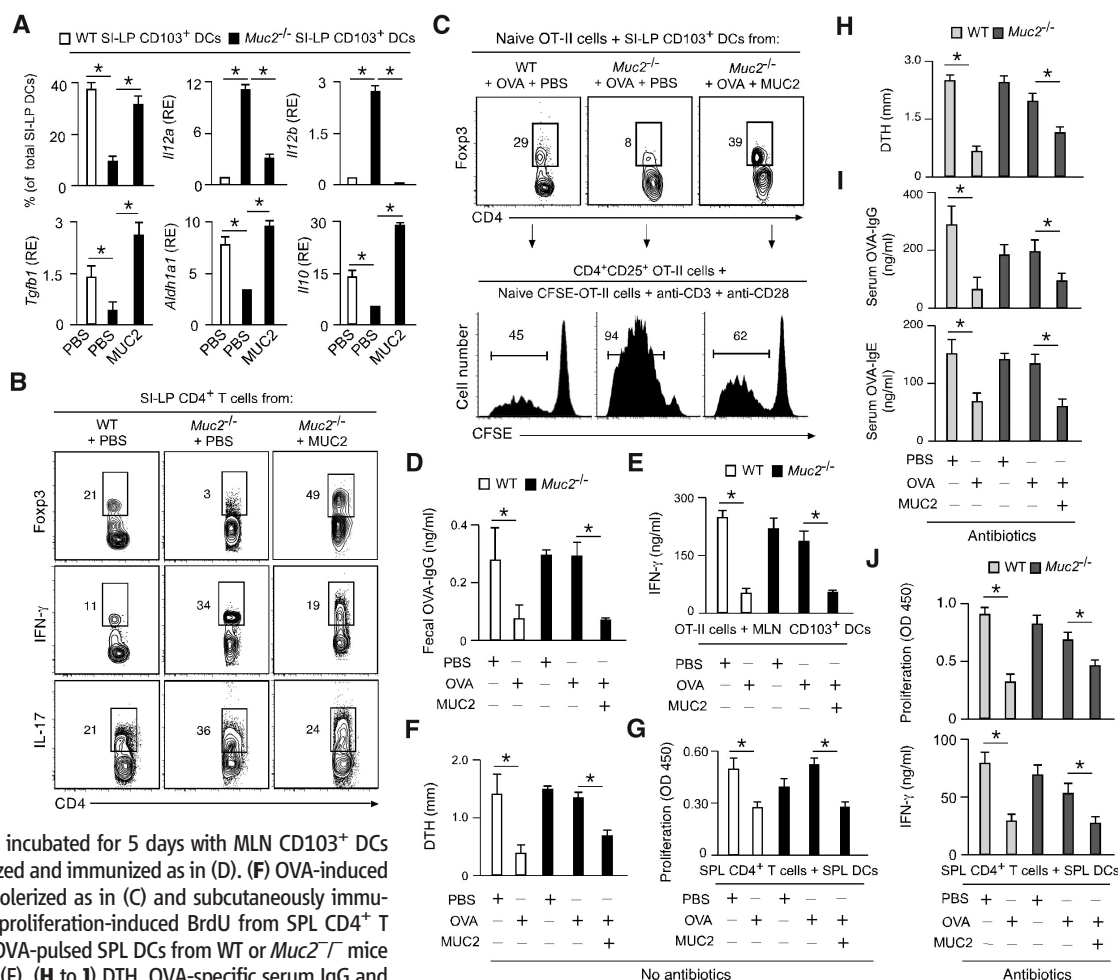
The immunoregulatory function of MUC2 was further explored in MUC2-deficient (*Muc2*^{-/-}) mice (12). Compared to wild-type controls, *Muc2*^{-/-} mice showed more IEC-adherent bacteria and

their SI-LP CD103⁺ and CX3CR1⁺ DCs expressed more TNF and IL-12, but less IL-10, TGF- β 1, and ALDH1A1 and thus induced fewer T_{reg} cells (Fig. 2, B and C, and fig. S12A). Accordingly, *Muc2*^{-/-} mice had fewer SI-LP T_{reg} cells but more proinflammatory T_H1 and IL-17-producing T_H17 cells (fig. S12B). These changes were associated with increased bacteria-bound IgA Abs and SI-LP DC expression of B cell-activating factor of the TNF family (BAFF) and a proliferation-inducing ligand (APRIL) (fig. S12, C and D), two IgA-inducing cytokines induced by microbial and inflammatory signals (3). Compared to wild-type mice, *Muc2*^{-/-} mice sterilized of gut bacteria normalized SI-LP DC expression of BAFF and APRIL, but neither augmented SI-LP DC expression of IL-10, TGF- β 1, and ALDH1A2 nor increased SI-LP T_{reg} cells (Fig. 2E and fig. S12, D and E). Thus, perturbations of gut homeostasis in *Muc2*^{-/-} mice cannot be solely ascribed to increased IEC-adherent bacteria.

We then verified whether MUC2 regulates IECs. Compared to controls, IECs from *Muc2*^{-/-} mice expressed less IL-10, TGF- β 1, ALDH1A1, and thymic stromal lymphopoietin (TSLP) (fig. S13A),

Fig. 3. MUC2 enhances gut homeostasis and oral tolerance.

(A) FC of CD103 and qRT-PCR of *Il12a*, *Il12b*, *Il10*, *Aldh1a1*, and *Tgfb1* in SI-LP CD103⁺ DCs from WT or *Muc2*^{-/-} mice gavaged for 5 days with phosphate-buffered saline (PBS) or MUC2. RE, relative expression compared to *Gapdh*. (B) FC of Foxp3, IFN- γ , IL-17, and CD4 in SI-LP T cells from WT or *Muc2*^{-/-} mice treated as in (A). (C) FC of Foxp3 and CD4 in naïve OT-II cells cultured for 5 days with SI-LP CD103⁺ DCs from WT or *Muc2*^{-/-} mice treated as in (A) and intragastrically immunized with OVA. CD4⁺CD25⁺ OT-II cells from these cultures were incubated for 5 days with CFSE-labeled naïve OT-II cells and Abs to CD3 and CD28; divided CFSElow cells were quantified by FC. (D) ELISA of fecal OVA-specific IgG from WT and *Muc2*^{-/-} mice tolerized with PBS, OVA, or OVA plus MUC2 for 5 days and immunized as in (C). (E) ELISA of IFN- γ from OT-II cells incubated for 5 days with MLN CD103⁺ DCs from WT or *Muc2*^{-/-} mice tolerized and immunized as in (D). (F) OVA-induced DTH in WT or *Muc2*^{-/-} mice tolerized as in (C) and subcutaneously immunized with OVA. (G) ELISA of proliferation-induced BrdU from SPL CD4⁺ T cells activated for 5 days with OVA-pulsed SPL DCs from WT or *Muc2*^{-/-} mice tolerized and immunized as in (F). (H to J) DTH, OVA-specific serum IgG and IgE, and SPL CD4⁺ T cell proliferation and IFN- γ secretion in WT or *Muc2*^{-/-} mice immunized and tolerized as in (F) after oral antibiotics. Data summarize two experiments with ≥ 4 mice per group (error bars, SD; unpaired Student's *t* test, **P* < 0.05) or show one of four experiments with similar results.



which generates tolerogenic DCs (13, 14). Gut sterilization failed to restore wild-type-like amounts of IL-10, ALDH1A1, and TSLP, but did normalize TGF- β 1 and RegIII γ (fig. S13A), an antimicrobial protein induced by bacteria (15). In humans, IECs expressed more TSLP in response to MUC2 (fig. S13A), which confirmed the tolerogenic function of MUC2 on IECs.

The immune and barrier functions of MUC2 were further uncoupled by gavaging *Muc2*^{-/-} mice with MUC2 from wild-type controls. When exposed to MUC2, SI-LP CD103⁺ and CX3CR1⁺ DCs from *Muc2*^{-/-} mice reduced OVA-specific CD4⁺ T cell proliferation and IFN- γ production, as did SI-LP DCs from wild-type mice (Fig. 2F). Gavaged MUC2 did not restore a visible barrier, but its capture not only augmented IL-10, TGF- β , and ALDH1A1 and decreased IL-12 in SI-LP DCs, but also increased SI-LP T_{reg} cells and reduced SI-LP T_H1 and T_H17 cells (Fig. 3, A and

B, and fig. S14A). Similarly, gavaged MUC2 helped SI-LP CD103⁺ DCs from *Muc2*^{-/-} mice to induce more OVA-specific T_{reg} cells after intragastric OVA immunization (Fig. 3C).

Notably, gavaged MUC2 enhanced the resistance of *Muc2*^{-/-} mice to dextran sodium sulfate (DSS), a colitogenic agent that disrupts the epithelial barrier. Although unable to attenuate inflammation-induced shortening of the LI, gavaged MUC2 ameliorated both clinical symptoms and histological lesions in DSS-treated *Muc2*^{-/-} mice, which showed less weight loss than wild-type or *Muc2*^{-/-} mice challenged with DSS in the absence of exogenous MUC2 (fig. S14, B to D). Thus, MUC2 attenuates bacteria-induced gut inflammation by delivering DC and IEC conditioning signals.

MUC2 Promotes Oral Tolerance

Oral tolerance consists of the attenuation of T and B cell responses to an antigen by prior oral ad-

ministration of that antigen and involves MLN induction of T_{reg} cells by migratory CD103⁺ DCs (2). Wild-type mice gavaged and later intragastrically immunized with OVA showed decreased gut B cell production of OVA-specific IgG Abs, which correlated with reduced IFN- γ production by OVA-reactive CD4⁺ T cells in response to OVA-pulsed MLNs, PPs, or SI-LP CD103⁺ DCs (Fig. 3, D and E, and fig. S15, A and B).

Tolerization also reduced OVA-induced delayed-type hypersensitivity (DTH)—an inflammatory skin reaction involving T_H1 cells—as well as DC-dependent splenic CD4⁺ T cell proliferation and IFN- γ secretion following systemic immunization of *Muc2*^{-/-} mice with OVA (Fig. 3, F and G, and fig. S15, C and D). Unlike wild-type mice, *Muc2*^{-/-} mice gavaged with OVA developed neither intestinal nor systemic tolerance (Fig. 3, D to G, and fig. S15, A to D). However, *Muc2*^{-/-} mice restored oral tolerance when gavaged with OVA in

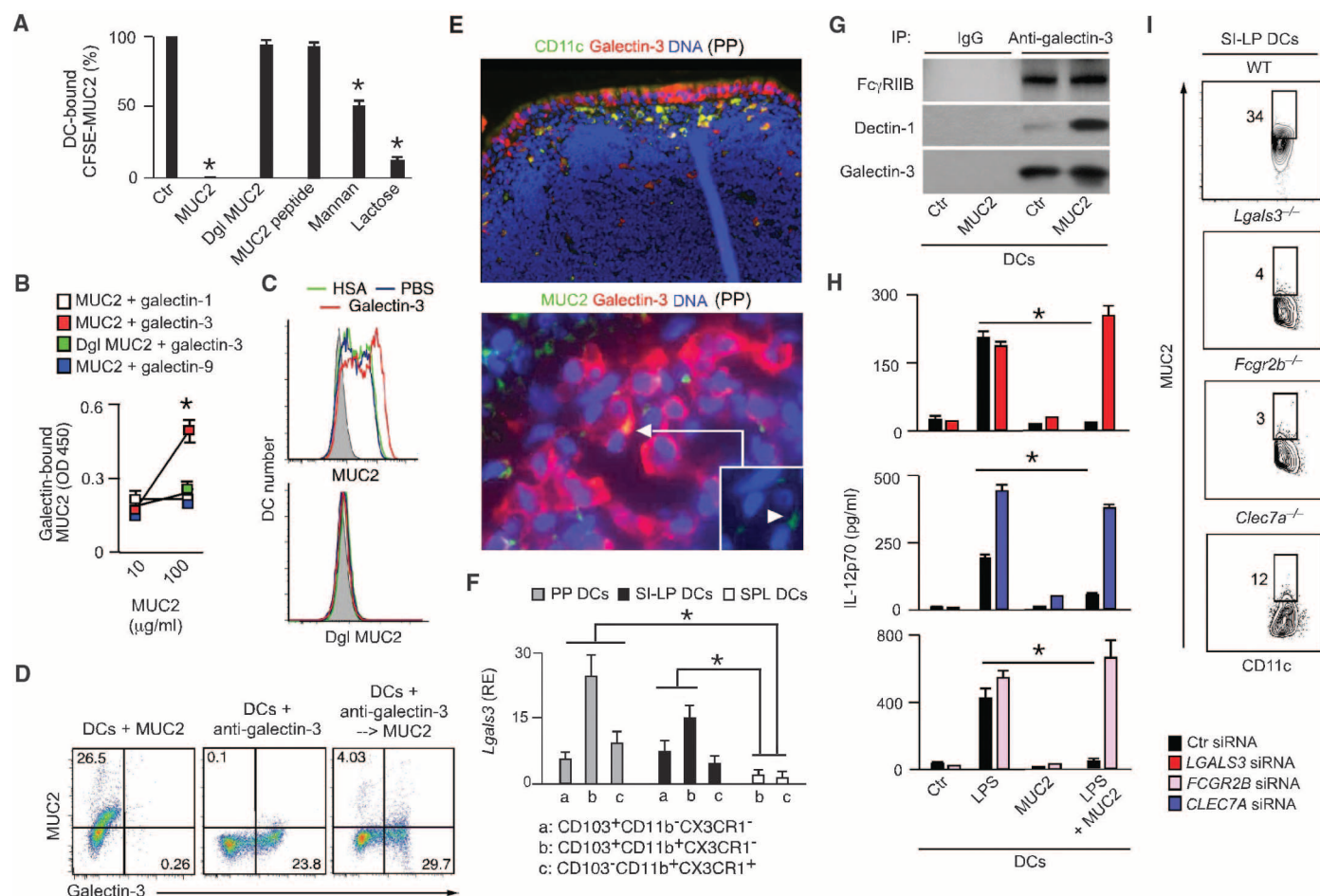


Fig. 4. MUC2 binds galectin-3, Dectin-1, and Fc γ RIIB on DCs. (A) FC of CFSE-MUC2 on human DCs preincubated with unlabeled native MUC2, deglycosylated (dgl) MUC2, MUC2 peptide, mannan, or lactose. Percent of MUC2 binding compared to medium alone. (B) ELISA of native or dgl MUC2 interaction with galectins. (C) CFSE-MUC2 or CFSE-dgl MUC2 binding to DCs preincubated with PBS, human serum albumin (HSA), or galectin-3. (D) CFSE-MUC2 binding to DCs before and after preincubation with a fluorescent Ab to galectin-3. (E) IFA of CD11c, galectin-3, Muc2, and DAPI in mouse PP sections. Original magnification, $\times 5$ (upper panel) and $\times 63$ (bottom panel). (F) qRT-PCR of mRNA for galectin-3 in DC subsets from mouse PPs, SI-LP, and SPL. RE,

relative expression compared to *Gapdh*. (G) Immunoprecipitation (IP) with control or anti-galectin-3 Abs of proteins from human DCs treated without (ctr) or with MUC2 for 30 min, followed by immunoblotting of Fc γ RIIB, Dectin-1, and galectin-3. (H) ELISA of IL-12p70 from human DCs exposed to scrambled (ctr) or *LGALS3* (galectin-3), *FCGR2B* (Fc γ RIIB), or *CLEC7A* (Dectin-1) small interfering RNAs (siRNAs) and cultured with or without LPS and/or MUC2 for 2 days. (I) Binding of CFSE-MUC2 to SI-LP DCs from WT, *Lgals3*^{-/-}, *Clec7a*^{-/-}, or *Fcgr2b*^{-/-} mice. Data summarize experiments with three donors or three mice from each strain (error bars, SD; unpaired *t* test, **P* < 0.05) or show one of three experiments with similar results.

the presence of MUC2 (Fig. 3, D to G, and fig. S15, A to D). The impairment of tolerance in *Muc2*^{-/-} mice was not merely due to bacteria-induced inflammation, as *Muc2*^{-/-} mice lacking gut bacteria did not attenuate OVA-specific DTH, IgG, and IgE responses after gavage with OVA (Fig. 3, H to J). Yet, tolerance was restored when OVA was gavaged in combination with MUC2 (Fig. 3, H to J). Thus, mucus actively constrains the immunostimulating properties of an oral soluble antigen.

MUC2 Binds a Galectin-3–Dectin-1–FcγRIIB Receptor Complex on DCs

Carbohydrates account for 80% of the weight of MUC2 (1) and may thus mediate its binding to DCs. Indeed, unlabeled glycosylated MUC2 inhibited the binding of CFSE-labeled native MUC2 to human DCs, whereas unlabeled deglycosylated MUC2 or a MUC2 peptide did not (Fig. 4A and fig. S16A). C-type lectin receptors (CLRs) and soluble galectins form carbohydrate-binding DC

platforms with tolerogenic function (16–20). Saturation of CLRs and galectins with mannan and lactose, respectively, attenuated MUC2 binding to DCs (Fig. 4A). DCs express galectin-1, -3 and -9 (21), but only galectin-3 interacted with glycosylated MUC2 and increased its binding to DCs through a process that was inhibited by MUC2 deglycosylation or Ab-mediated blockade of galectin-3 (Fig. 4, B to D). In agreement with their ability to bind galectin-3 following its

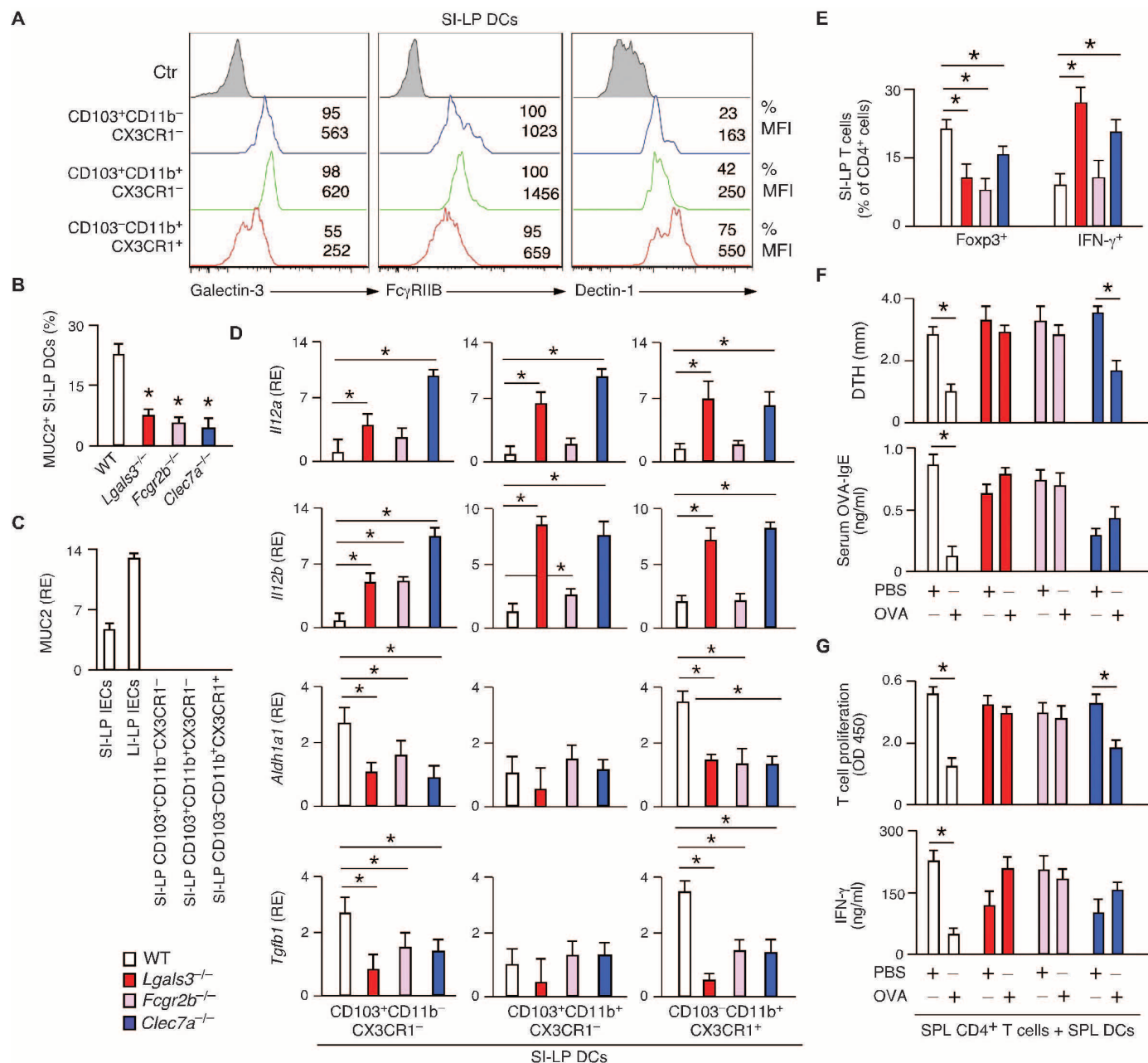


Fig. 5. Galectin-3, Dectin-1, and FcγRIIB enhance gut homeostasis and oral tolerance. (A) FC of galectin-3, FcγRIIB, and Dectin-1 on SI-LP DCs from WT mice. Galectin-3 was measured in permeabilized DCs. MFI, mean fluorescence intensity. (B) Quantification of MUC2⁺ SI-LP DCs from WT, *Lgals3*^{-/-}, *Clec7a*^{-/-}, or *Fcgr2b*^{-/-} mice by IFA of 10 to 12 SI-LP sections per group. (C) qRT-PCR of *Muc2* in IECs and SI-LP DCs from WT mice. RE, relative expression compared to *Gapdh*. (D and E) qRT-PCR of *Il12a*, *Il12b*, *Aldh1a1*, and *Tgfb1* in SI-LP DCs and FC of Foxp3 and IFN-γ in SI-LP CD4⁺ T cells from WT, *Lgals3*^{-/-},

Clec7a^{-/-}, or *Fcgr2b*^{-/-} mice. RE, relative expression compared to *Gapdh*. (F) DTH and ELISA of OVA-specific IgE in WT, *Lgals3*^{-/-}, *Clec7a*^{-/-}, or *Fcgr2b*^{-/-} mice intragastrically tolerized with PBS or OVA for 5 days and subcutaneously immunized with OVA. (G) ELISA of proliferation-induced BrdU from SPL CD4⁺ T cells activated for 5 days by OVA-pulsed SI-LP DCs from WT, *Lgals3*^{-/-}, *Clec7a*^{-/-}, or *Fcgr2b*^{-/-} mice tolerized and immunized as in (F). Data summarize two experiments with ≥4 mice per group (error bars, SD; unpaired Student's *t* test, **P* < 0.05) or show one of four experiments with similar results.

secretion (16, 21), DCs increased both soluble and membrane-bound galectin-3 in response to LPS (fig. S16B). Thus, DCs may form a galectin-3-based MUC2-binding platform after sensing gut microbial signals.

Accordingly, PP and SI-LP CD103⁺ and CX3CR1⁺ DCs produced more galectin-3 than splenic DCs did and showed intracellular galectin-3 colocalized with MUC2 (Fig. 4, E and F, and fig. S16, C to E). SI-LP DCs also displayed more surface galectin-3 (Fig. 4F and fig. S16F), part of which may have an epithelial origin (21). Indeed, galectin-3 was detected in both IECs and GCs, and its secretion augmented upon IEC exposure to bacteria (fig. S16, G and H). Consequently, DCs acquired more surface galectin-3 after exposure to IEC supernatant or migration across IECs (fig. S16, I and J).

We then determined how soluble galectin-3 anchors itself and MUC2 to DCs. Galectin-3 binds Dectin-1, a CLR that induces tolerogenic DCs and enhances gut homeostasis by recognizing fungal carbohydrates (16, 17, 20). Dectin-1 further interacts with the anti-inflammatory receptor FcγRIIB in response to glycans from therapeutic IgG Abs (22). In human embryonic kidney 293

cells, MUC2 bound transfected Dectin-1 and FcγRIIB in the presence of galectin-3 (fig. S17, A and B). In DCs, MUC2 binding correlated with increased surface amounts of galectin-3, Dectin-1, and FcγRIIB and caused Dectin-1 recruitment to a preformed galectin-3–FcγRIIB complex (Fig. 4G and fig. S17C). Accordingly, DCs lacking galectin-3, Dectin-1, or FcγRIIB neither effectively bound MUC2 nor decreased IL-12 production in response to MUC2 (Fig. 4, H and I, and fig. S17, D to G).

In wild-type mice, SI-LP CD103⁺ DCs expressed less Dectin-1 but more galectin-3 and FcγRIIB than CX3CR1⁺ DCs did (fig. 5A). Compared to *Lgals3* (galectin-3)^{-/-}, *Clec7a* (Dectin-1)^{-/-}, or *Fcgr2b* (FcγRIIB)^{-/-} mice, wild-type mice had a comparable SI mucus layer, but showed more SI-LP DCs containing MUC2 (Fig. 5B and fig. S18, A and B). Unlike IECs, these SI-LP DCs did not express MUC2 (Fig. 5C), which confirmed that gut DCs acquire MUC2 from the external environment. Compared to controls, *Lgals3*^{-/-}, *Clec7a*^{-/-}, or *Fcgr2b*^{-/-} mice had SI-LP DCs that produced more IL-12, but less ALDH1A1 and TGF-β1 and did not respond to MUC2 (Fig. 5D and fig. S19). These mice also had fewer SI-LP

T_{reg} cells, more SI-LP T_H1 cells, and impaired tolerogenic T and B cell responses to OVA (Fig. 5, E to G). Thus, MUC2 may enhance gut homeostasis and tolerance by assembling a signal-transducing Dectin-1–FcγRIIB complex on DCs with the help of soluble galectin-3.

MUC2 Tolerizes DCs by Inducing β-Catenin

How do Dectin-1 and FcγRIIB convey tolerogenic signals to DCs exposed to MUC2? Dectin-1 phosphorylates AKT (23) and may thus induce β-catenin, an AKT-regulated transcription factor required by gut tolerogenic DCs (24). Indeed, MUC2 alone or combined with LPS phosphorylated AKT and glycogen synthase kinase-3β (GSK-3β) (Fig. 6A), two events that cause GSK-3β inactivation and inhibition of β-catenin degradation (24, 25). Consequently, MUC2 elicited cytoplasmic accumulation and nuclear translocation of β-catenin in wild-type but not *Clec7a*^{-/-} DCs (Fig. 6A and fig. S20, A and B).

In some tumors, β-catenin interacts with nuclear factor κB (NF-κB) p65 to impede the binding of activating p50-p65 complexes to death-inducing genes (25). In DCs, MUC2 alone or coupled with LPS enhanced nuclear β-catenin interaction with NF-κB p65 and decreased NF-κB p50-p65 binding

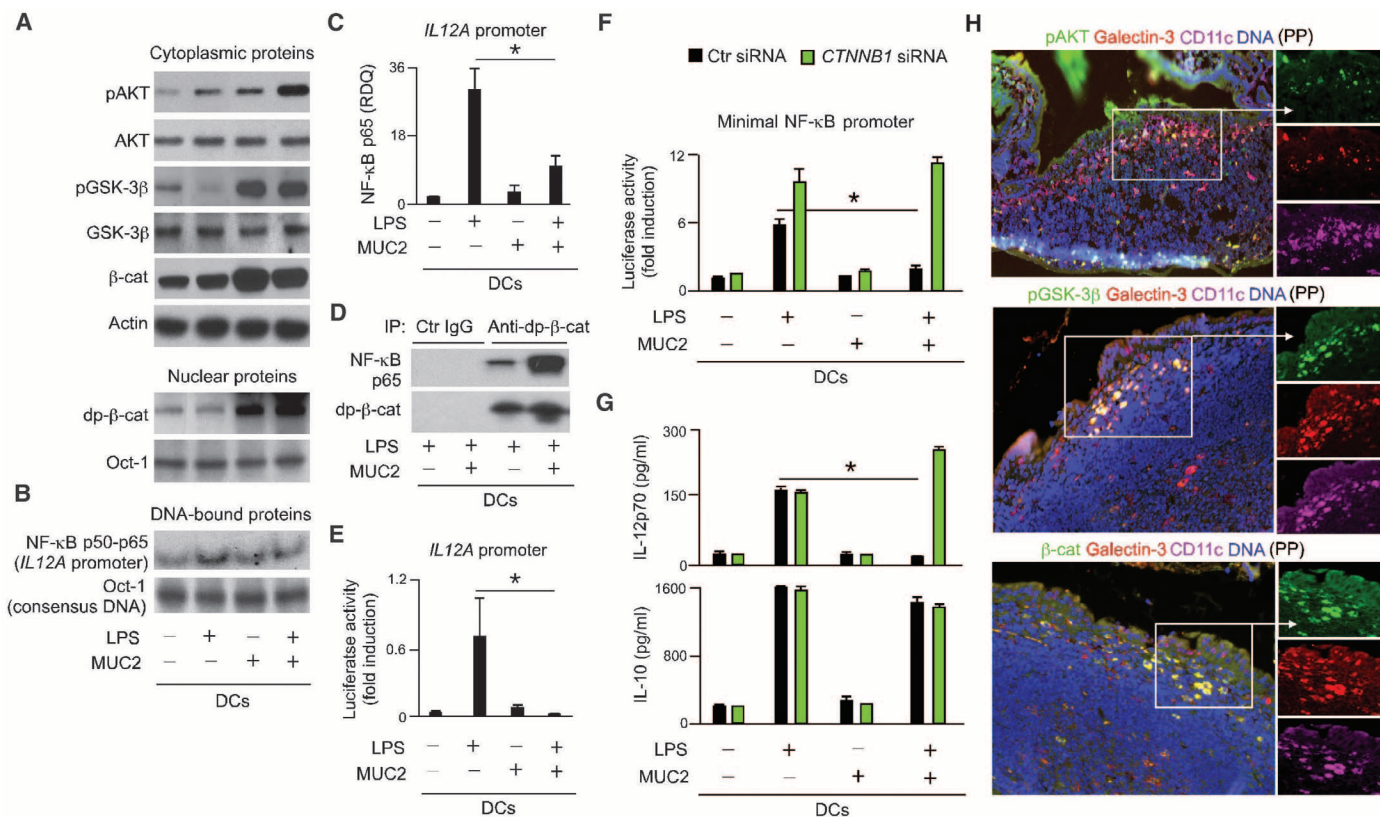


Fig. 6. MUC2 impairs NF-κB-driven inflammatory signals via β-catenin.

(A) WB of cytoplasmic or nuclear phospho (p)-AKT, AKT, pGSK-3β, GSK-3β, β-catenin (β-cat), dephospho (dp)-β-cat, actin, and octamer-1 (Oct-1) from human DCs cultured with or without LPS and/or MUC2 for 10 min. (B) Electrophoretic mobility gel shift assay of IL12A-bound NF-κB p65-p50 and consensus DNA-bound Oct-1 in DCs cultured as in (A) for 3 hours. (C) Chromatin IP of IL12A-bound NF-κB p65 in DCs cultured as in (A) for 3 hours. RDQ, relative DNA quantity. (D) IP with control (ctr) IgG Ab or anti-dp-β-cat Ab of nuclear

proteins from DCs cultured with or without LPS and/or MUC2 for 10 min, followed by WB of NF-κB p65 and dp-β-cat. (E) IL12A transcription in DCs cultured as in (A) for 2 days. (F and G) NF-κB-mediated transcription and ELISA of IL-12p70 and IL-10 in DCs cultured as in (E) in the presence of scrambled (ctr) or *CTNNB1* (β-cat) siRNAs. (H) IFA of pAKT, pGSK-3β, β-cat, galectin-3, CD11c, and DAPI in mouse PPs. Original magnification, ×5. Data show one of three experiments yielding similar results or summarize three experiments (error bars, SD; unpaired *t* test, **P* < 0.05).

to both minimal and *IL12* gene promoters, thus impairing their transcription (Fig. 6, B to E, and fig. S20C). Accordingly, β -catenin deficiency impeded MUC2-mediated inhibition of LPS-induced IL-12 but not IL-10 transcription and/or secretion, whereas β -catenin overexpression dampened TNF-induced NF- κ B-driven transcription (Fig. 6, F and G, and fig. S20, D to F). Consistent with these data, galectin-3-expressing DCs from PPs contained abundant β -catenin in addition to activated AKT and inactive GSK-3 β (Fig. 6H).

Besides AKT, Dectin-1 phosphorylates SYK, which activates NF- κ B through a pathway mitigated by Fc γ RIIB via SH2 domain-containing inositol 5-phosphatase-1 (SHIP-1) (22, 26). SYK also activates cAMP responsive element binding protein (CREB), a calcium-dependent IL-10-inducing protein that removes the co-activator CREB-binding protein (CBP) from DNA-bound NF- κ B (27, 28). Besides triggering SYK and SHIP-1 phosphorylation, MUC2 alone or combined with LPS decreased NF- κ B p65 but not p50 nuclear translocation and increased galectin-dependent calcium fluxes, phosphorylation of CREB-targeting AKT, ERK1/2 and p38 kinases, phosphorylation and nuclear translocation of CREB, binding of CREB to *IL10* and *IL12* promoters, and loss of CBP from the *IL12* promoter (figs. S20G and S21, A to G). Thus, similar to hyperglycosylated IgG Abs used to treat autoimmune disorders (22), MUC2 may recruit SHIP-1 via Fc γ RIIB to constrain proinflammatory NF- κ B but not tolerogenic CREB signals emanating from Dectin-1 and SYK. In addition to inducing IL-10, CREB may cooperate with Dectin-1-induced β -catenin to inhibit NF- κ B-dependent IL-12 production.

Conclusions

We have shown here that MUC2 enhances gut homeostasis and oral tolerance by conditioning DCs and IECs. Antigen-sampling DCs assemble galectin-3, Dectin-1, and Fc γ RIIB to acquire MUC2 across IECs and possibly from GCs (fig. S22A). This MUC2 receptor complex suppresses inflammatory but not tolerogenic DC responses by inhibiting NF- κ B via β -catenin (fig. S22B). How DCs tune down these signals during infection remains unclear, but pathogen-induced perturbations of MUC2 glycosylation and polymerization patterns may be involved (1). A full understanding of the immunoregulatory function of MUC2 could help to devise better vaccines and treatments against infections and food allergies and to unravel how alterations of MUC2 and its receptors aggravate inflammatory bowel disease (1, 20), thus leading to safer therapies against this disorder.

References and Notes

1. M. E. Johansson, J. M. Larsson, G. C. Hansson, *Proc. Natl. Acad. Sci. U.S.A.* **108** (suppl. 1), 4659–4665 (2011).
2. J. L. Coombes, F. Powrie, *Nat. Rev. Immunol.* **8**, 435–446 (2008).
3. A. Cerutti, K. Chen, A. Chorny, *Annu. Rev. Immunol.* **29**, 273–293 (2011).
4. M. Rescigno *et al.*, *Nat. Immunol.* **2**, 361–367 (2001).
5. J. H. Niess *et al.*, *Science* **307**, 254–258 (2005).
6. J. Farache *et al.*, *Immunity* **38**, 581–595 (2013).
7. J. L. Coombes *et al.*, *J. Exp. Med.* **204**, 1757–1764 (2007).
8. M. Bogunovic *et al.*, *Immunity* **31**, 513–525 (2009).
9. J. R. McDole *et al.*, *Nature* **483**, 345–349 (2012).
10. C. I. Yu *et al.*, *Immunity* **38**, 818–830 (2013).
11. D. Mucida *et al.*, *Science* **317**, 256–260 (2007).
12. A. Velcich *et al.*, *Science* **295**, 1726–1729 (2002).
13. I. D. Iliev, E. Mileti, G. Matteoli, M. Chieppa, M. Rescigno, *Mucosal Immunol.* **2**, 340–350 (2009).
14. M. Rimoldi *et al.*, *Nat. Immunol.* **6**, 507–514 (2005).

15. S. Vaishnava *et al.*, *Science* **334**, 255–258 (2011).
16. A. Esteban *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **108**, 14270–14275 (2011).
17. S. Dillon *et al.*, *J. Clin. Invest.* **116**, 916–928 (2006).
18. J. M. Ibarregui *et al.*, *Nat. Immunol.* **10**, 981–991 (2009).
19. Y. Zhou *et al.*, *Nat. Med.* **16**, 1128–1133 (2010).
20. I. D. Iliev *et al.*, *Science* **336**, 1314–1317 (2012).
21. G. A. Rabinovich, M. A. Toscano, *Nat. Rev. Immunol.* **9**, 338–352 (2009).
22. C. M. Karsten *et al.*, *Nat. Med.* **18**, 1401–1406 (2012).
23. K. M. Dennehy, J. A. Willment, D. L. Williams, G. D. Brown, *Eur. J. Immunol.* **39**, 1379–1386 (2009).
24. S. Manicassamy *et al.*, *Science* **329**, 849–853 (2010).
25. J. Deng *et al.*, *Cancer Cell* **2**, 323–334 (2002).
26. D. Strasser *et al.*, *Immunity* **36**, 32–42 (2012).
27. E. K. Kelly, L. Wang, L. B. Ivashkiv, *J. Immunol.* **184**, 5545–5552 (2010).
28. A. Y. Wen, K. M. Sakamoto, L. S. Miller, *J. Immunol.* **185**, 6413–6419 (2010).

Acknowledgments: This study was supported by the National Institute of Allergy and Infectious Diseases, NIH (AI61093, AI57653, AI95613, AI96187 and AI74378 to A.C. and AI073899, DK072201 and AI095245 to J.M.B.) and by Redes Temáticas de Investigación Cooperativa en Salud/Fondo Europeo de Desarrollo Regional (RD12/0036/0054 to A.B.). pRSETb-mRFP used for red bacteria is under a materials transfer agreement with R. Y. Tsien at the University of California, San Francisco, and Howard Hughes Medical Institute. The data presented in this manuscript are tabulated in the main paper and the supplementary materials.

Supplementary Materials

www.sciencemag.org/content/342/6157/447/suppl/DC1

Materials and Methods

Acknowledgments

Figs. S1 to S22

Tables S1 to S8

References (29–37)

Movies S1 and S2

18 March 2013; accepted 5 September 2013

Published online 26 September 2013;

10.1126/science.1237910

REPORTS

Observation of Floquet-Bloch States on the Surface of a Topological Insulator

Y. H. Wang,* H. Steinberg, P. Jarillo-Herrero, N. Gedik†

The unique electronic properties of the surface electrons in a topological insulator are protected by time-reversal symmetry. Circularly polarized light naturally breaks time-reversal symmetry, which may lead to an exotic surface quantum Hall state. Using time- and angle-resolved photoemission spectroscopy, we show that an intense ultrashort midinfrared pulse with energy below the bulk band gap hybridizes with the surface Dirac fermions of a topological insulator to form Floquet-Bloch bands. These photon-dressed surface bands exhibit polarization-dependent band gaps at avoided crossings. Circularly polarized photons induce an additional gap at the Dirac point, which is a signature of broken time-reversal symmetry on the surface. These observations establish the Floquet-Bloch bands in solids and pave the way for optical manipulation of topological quantum states of matter.

Three-dimensional topological insulators (TIs) host an exotic surface state that obeys the Dirac equation and exhibit spin-momentum

locking. The gapless surface states are protected by time-reversal symmetry (TRS), the breaking of which is predicted to lead to many exotic

phenomena (1–3). Doping TIs with magnetic impurities breaks TRS on the surface (4–7), but it also introduces disorder (8); the coherent interaction between light and matter is a promising alternative route toward such a broken symmetry phase (9–12). This coherent effect is seen in atoms and molecules as hybridized states distinctive in their energy spectra (13, 14) and in photonic systems as Floquet states (15). In solid-state systems, the photon-dressed bands lead to a periodic band structure in both energy and momentum called Floquet-Bloch states (16). In the case of TIs, an additional effect is expected to take place when circularly polarized light is coupled with the surface states: TRS will be spontaneously broken and the surface Dirac cone becomes gapped (9, 17).

Floquet theorem states that a Hamiltonian periodic in time has quasistatic eigenstates that are evenly spaced by the drive photon energy (18).

Department of Physics, Massachusetts Institute of Technology, Cambridge, MA 02139, USA.

*Present address: Department of Physics and Applied Physics, Stanford University, Stanford, CA 94305, USA.

†Corresponding author. E-mail: gedik@mit.edu

These so-called Floquet states can be regarded as a time analog of Bloch states, which are the eigenstates of a Hamiltonian periodic in space (19). Combining the two situations, a periodic excitation on a crystalline lattice induces Floquet-Bloch bands that repeat in both momentum and energy. Just as different Bloch bands hybridize and develop band gaps at the crossing points (19), the crossing points between different orders (n) of the Floquet-Bloch bands open dynamic gaps (16, 20). Although conventional optical

spectroscopy (21–23) and photoemission spectroscopy (24, 25) have shown many coherent phenomena under intense radiation, a technique that can probe the states in an energy-momentum-resolved manner while applying a strong photo-excitation is needed to image the Floquet-Bloch states and the photo-induced band gaps on the surface of a TI.

Time- and angle-resolved photoemission spectroscopy (TrARPES) is a powerful technique capable of resolving such photo-induced band gaps

(26–28). When the photon energy of the laser that excites the sample is below the bulk band gap (typically less than 300 meV), the coherent interaction between light and the TI surface states is the dominant effect (10, 11). To achieve this regime, we used polarized photons at midinfrared (MIR) wavelengths to investigate the photon-dressed surface states in TIs.

We used a time-of-flight spectrometer to simultaneously acquire the complete surface band structure of Bi₂Se₃ without changing the sample or

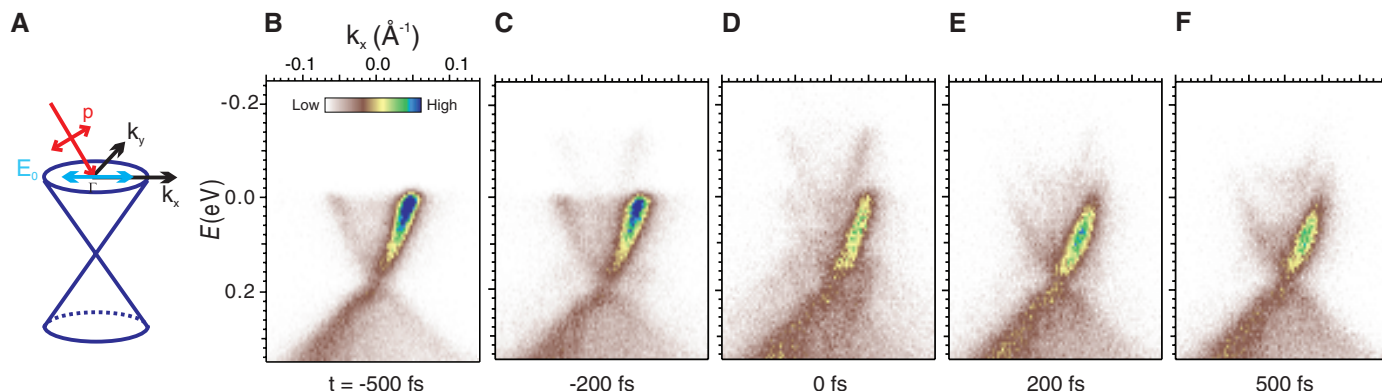
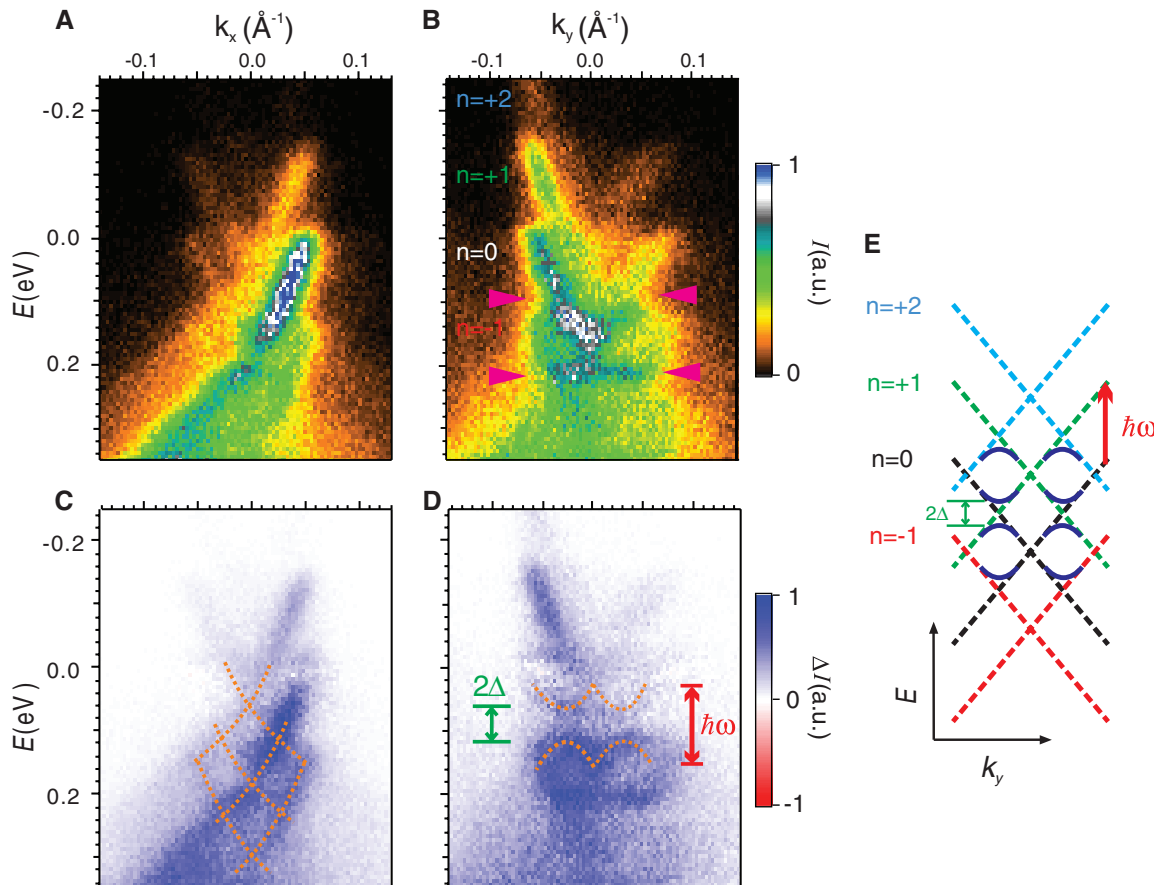


Fig. 1. Angle-resolved photoemission spectra (APRES) of Bi₂Se₃. (A) A sketch of the experimental geometry for the p-polarized case. k_x is defined to be the in-plane electron momentum parallel to the pump scatter-

ing plane. (B to F) ARPES data for several pump-probe time delays t (values indicated in the figure) under strong linearly polarized mid-infrared (MIR) excitation of wavelength $\lambda = 10 \mu\text{m}$.

Fig. 2. ARPES spectra at 0 fs time delay under linearly polarized MIR excitation. (A) and (B) show the energy-momentum spectra through Γ along the k_x and k_y directions, respectively. (C) and (D) are the same spectra after subtracting the spectra at $t = -500$ fs (35). Dashed orange lines are guides to the eye. (E) Sketch of the side bands of different order as induced by the mid-infrared excitation. Avoided crossing occurs along k_y , leading to a pattern of ∞ around the Dirac point. ω is the drive photon frequency, and Δ is the half gap at the avoided crossings.



the detector orientation in a pump-probe scheme (28, 29). The MIR pump pulses are generated from a commercial optical parametric amplifier pumped by a Titanium:Sapphire amplifier. The output pulses are tunable in wavelength from 4 μm to 17 μm with 1 μJ peak energy. The MIR pulses are focused to a 300- μm diameter spot [full width at half maximum (FWHM)] on the single-crystal Bi_2Se_3 sample at an angle of 45° . The pulsewidth is estimated to be 250 fs (FWHM) from the rising edge of the momentum-integrated time spectrum (fig. S1D). We estimated the amplitude of the electric field to be $E_0 = 2.5(\pm 1) \times 10^7$ V/m on the surface of the sample after taking into account the loss through the optics, the geometric effect, and the Fresnel reflection (31) at the vacuum-sample interface (31). The polarization of the MIR pulse is adjusted by a quarter wave-plate. Figure 1A illustrates the p-polarized case in which the electric field of light is in the plane of incidence. The component of the surface electron momentum in the plane of incidence is defined as k_x .

Figure 1, B to F, shows energy-momentum spectra of Bi_2Se_3 obtained at several time delays t after the intense linearly polarized MIR excitation. At $t = -500$ fs, the probe ultraviolet (UV) pulse is ahead of the MIR pulse and the band structure is similar to that of an unperturbed system (Fig. 1B) (28). The asymmetry in the spectral intensity

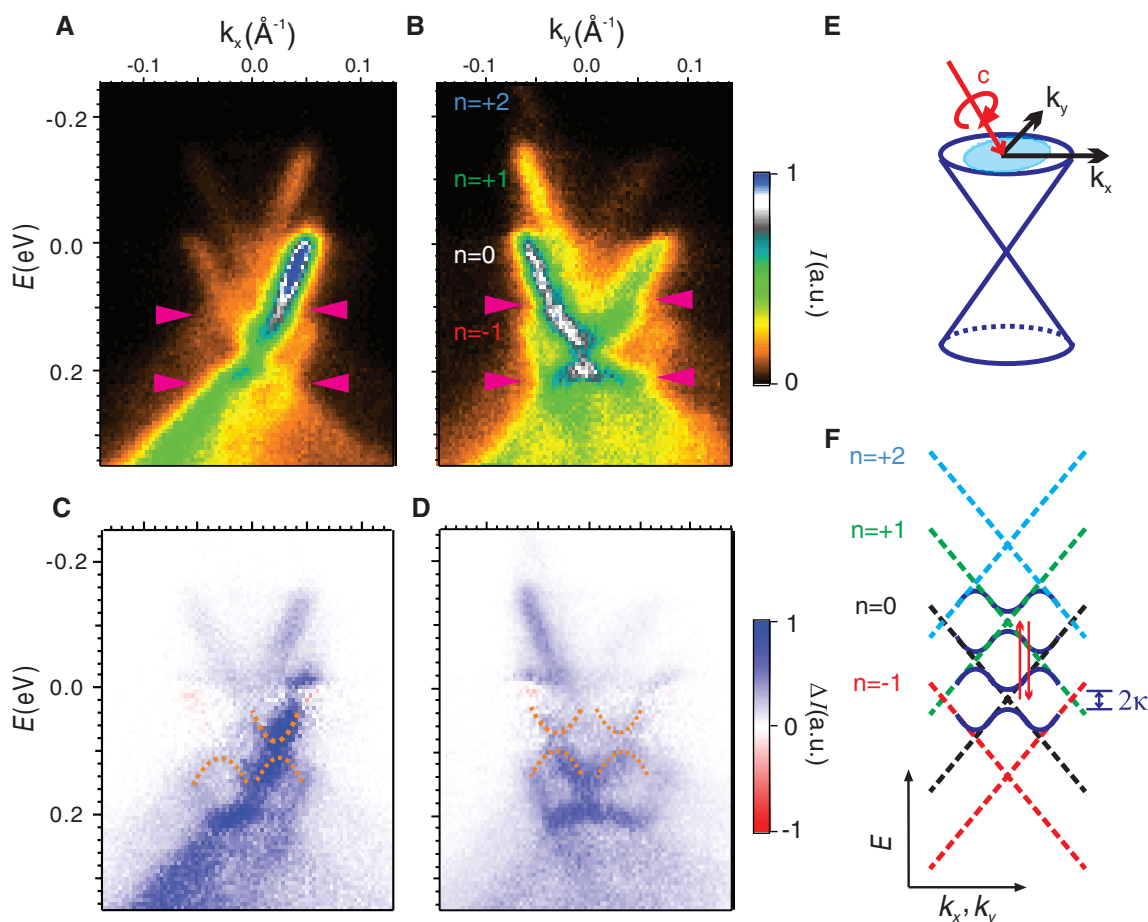
about zero momentum Γ is due to the coupling between the linearly polarized UV pulse and the spin-orbit texture of the surface states of TI (29). When the two pulses start to overlap in time ($t = -200$ fs), replicas of the surface Dirac cone appear above and below the original band (Fig. 1C). The energy difference between the bands equals exactly the pump photon energy of $\hbar\omega = 120$ meV, where $\hbar$ is the reduced Planck constant and ω is the angular frequency of light. The intensity of such side bands becomes stronger, whereas the original band gets weaker when the pump and probe pulses are completely overlapped at $t = 0$ (Fig. 1D). When the side bands become weaker again at 200 fs (Fig. 1E), the spectral intensity still above the Fermi level occupies the higher energy states of the original surface and bulk bands rather than the side bands. This suggests the onset of a thermally excited carrier population, as has been observed previously with 1.5-eV photoexcitations (26, 28). This excited population is still present at 500 fs (Fig. 1F), consistent with the observed photoexcited carrier cooling time in this material (26, 28). The fact that the side bands are only present during the time duration of the pump pulse suggests that it is a consequence of the coherent interaction between the MIR photons and the electron system. In a TrARPES experiment, a strong IR field may also generate band replicas through laser-assisted photoemission

(LAPE) (24, 25). Because this effect is caused by the absorption of pump photons by the near-free electrons in the final states of photoemission, the LAPE bands do not open band gaps where they cross, and their intensity is at a minimum in the direction perpendicular to the light polarization, which is inconsistent with our findings (31).

To see how the photon-dressed bands change with respect to the polarization of light and whether there are band gaps, we compared the energy-momentum spectra taken at $t = 0$ fs along the directions parallel and perpendicular to the electric field of the linearly polarized pump (Fig. 2). Compared with the side bands along k_x (Fig. 2A), the side bands in the k_y direction show a higher order ($n = +2$) more clearly (Fig. 2B). More important, the bands are much stronger and show features that deviate from a mere stacking of Dirac cones at energies close to the $n = 0$ Dirac point ($0.15 \text{ eV} < E < 0.3 \text{ eV}$). The otherwise linearly dispersing $n = 0$ cone becomes discontinuous and distorted at $E = 0.1 \text{ eV}$ and 0.22 eV (Fig. 2B, pink triangles).

To better visualize these features, we subtracted the spectrum taken at $t = -500$ fs from the spectrum at $t = 0$ fs, which includes the spectral contribution from the unperturbed surface bands due to the finite pulse width (31). The difference spectrum along k_y (Fig. 2D) shows a pattern resembling an ∞ sign centered at the $n = 0$

Fig. 3. ARPES spectra at 0 fs time delay under circularly polarized MIR excitation. (A to D) are analogous to the corresponding panels in Fig. 2. **(E)** The projection of the electric field on the surface plane (light blue) is elliptical, and the avoided crossing appears along both directions. The chirality allows emitting and absorbing a photon to open a band gap at the Dirac point as sketched in the side-band diagram in **(F)**. κ is half of the band gap at the Dirac point.



Dirac point. There is a replica of this ∞ pattern at one pump photon energy (120 meV) above the one around $n = 0$ Dirac point, suggesting that this is a coherent feature on the photo-induced bands. $E = 0.1$ eV is the middle point of these two ∞ , suggesting that the kinks in the raw spectrum (Fig. 2B) correspond to opening of band gaps. These features are in contrast with the difference spectrum in the k_x direction, where the pattern is simply composed of three Dirac cones shifted by $\hbar\omega$ (Fig. 2C). This momentum-dependent feature is consistent with the prediction of photon-dressed Dirac bands in graphene under linearly polarized light (32–34), where Floquet-Bloch bands open band gaps along the direction perpendicular to the electric field, whereas the bands with momentum parallel to the field remain gapless.

For an ideal Dirac cone (Fig. 2E), the avoided crossing between adjacent orders is centered at momenta $\pm\omega/2v$, where v is the Fermi velocity of the surface states. In our case, we can see in the difference spectra the crossing of bands at $k_y \sim \pm 0.03 \text{ \AA}^{-1}$ (Fig. 2C), whereas the band gaps appear at similar values of k_y (Fig. 2D). This is slightly bigger than the expected value of $\omega/2v = 0.02 \text{ \AA}^{-1}$ [using $\hbar\omega = 120 \text{ meV}$ and $\hbar v = 3 \text{ eV\AA}$ (Fig. 1A)], likely resulting from the reduced velocity of bands below the Dirac point (Fig. 1). At $k_y = \pm 0.06 \text{ \AA}^{-1}$, where $n = \pm 1$ cross, there is no resolvable gap (Fig. 2D). Therefore, the ∞

pattern around the $n = 0$ Dirac point is a result of the crossings and avoided crossings between $n = 0$ and $n = \pm 1$ (Fig. 2, D and E). The energy distribution curve (fig. S1A) through the avoided crossing shows a 2Δ value of 62 meV. Along with the crossings and avoided crossings between different orders, the gap size is consistent with the theory of photon-dressed Dirac systems based on the Floquet picture (31–34).

Having shown that linearly polarized MIR photons couple with the surface states into Floquet-Bloch bands, we investigated how these bands change when the photons become circularly polarized. Unlike the bands under linearly polarized light, the spectrum under circularly polarized light shows avoided crossings at $\sim \pm 0.03 \text{ \AA}^{-1}$ along both k_x and k_y (Fig. 3, A to D). Spectral cuts along other in-plane directions through Γ (fig. S3C) further show that the dynamical gap does not close for any direction. This is consistent with the Floquet-Bloch bands generated by the coupling between circularly polarized in-plane electric field and the surface states (Fig. 3E).

Knowing that the surface electrons and the rotating electric field of the circularly polarized MIR pulse are coupled, we examined the Dirac point of the induced Floquet-Bloch bands for signatures of TRS breaking (Fig. 3F). The spectral weight above and below the Dirac point increases at $t = 0$ for both polarizations (Figs. 2, C and

D, and 3, C and D). To be able to resolve any gap around the Dirac point, we converted the intensity spectra for both polarizations (Figs. 2B and 3B) into energy distribution curves (EDCs) by integrating the spectra over a small momentum window ($0.005 \text{ \AA}^{-1}$) at each momentum point along k_y (Fig. 4). The EDCs show that only the spectrum obtained under circularly polarized excitations opens a band gap at the Dirac point (Fig. 4A), whereas such a gap is absent in the curves taken under linearly polarized light (Fig. 4B). The EDC through the Γ point under circularly polarized light clearly show two peaks at the Dirac point (Fig. 4C, blue). [The small hump in the EDC under linearly polarized excitation (Fig. 4C, red) is a result of the finite momentum resolution of our spectrometer ($\sim 0.005 \text{ \AA}^{-1}$) and is also visible in the EDC of unperturbed spectrum (Fig. 4C, black).] This result is similarly observed in the difference spectrum between the two polarizations as negative spectral weight at the Dirac point (fig. S4A). By fitting the EDC through the Γ point in Fig. 4A, we obtained the band gap $2\kappa = 53 \text{ meV}$ (fig. S1B). Using the 2Δ value we obtained from the dynamic gap (31), we found that the Dirac gap is consistent with the gap resulting from the two-photon process (absorbing and emitting a photon) that is only allowed under circular polarization that breaks TRS (9, 34).

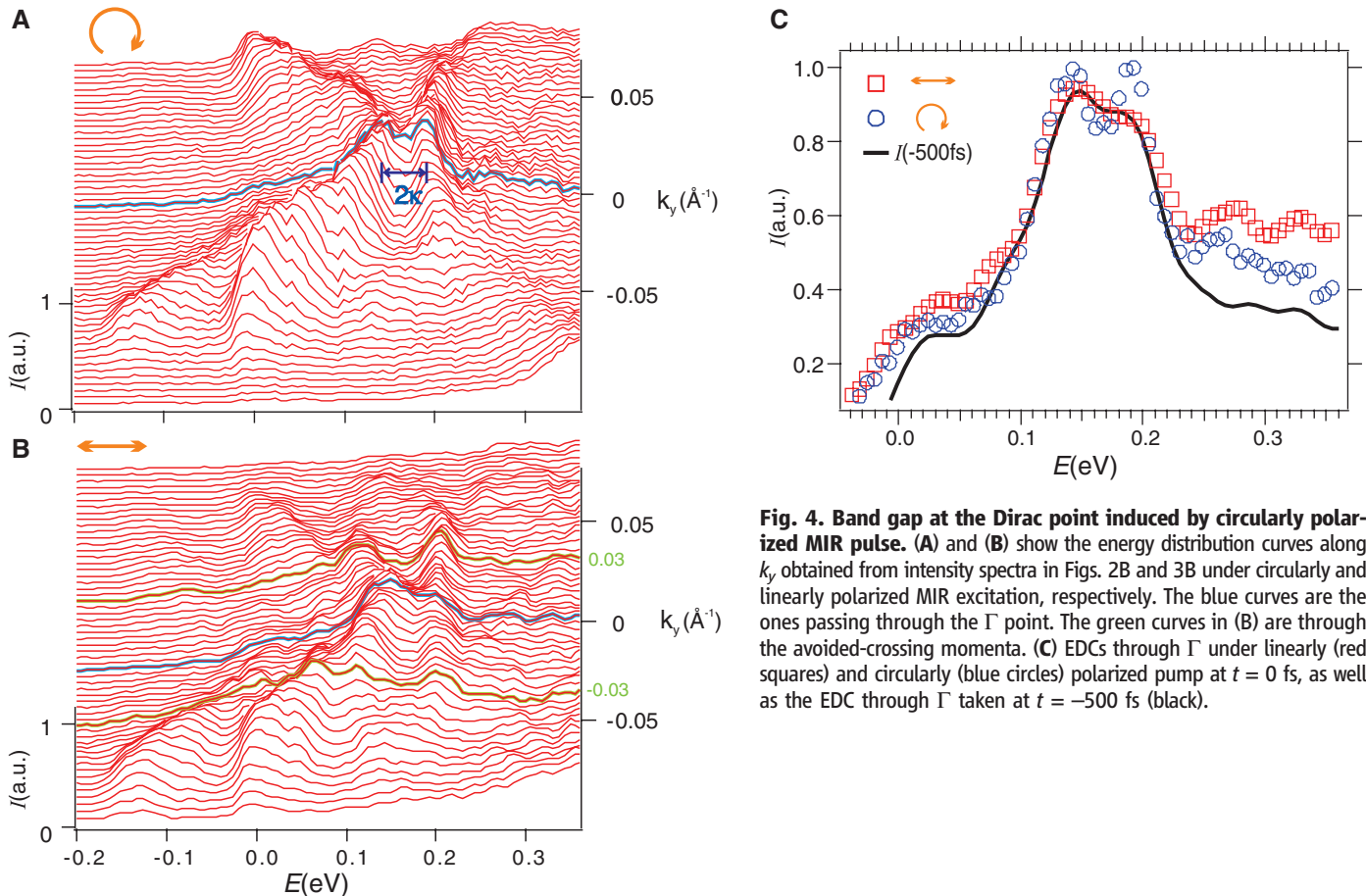


Fig. 4. Band gap at the Dirac point induced by circularly polarized MIR pulse. (A) and (B) show the energy distribution curves along k_y obtained from intensity spectra in Figs. 2B and 3B under circularly and linearly polarized MIR excitation, respectively. The blue curves are the ones passing through the Γ point. The green curves in (B) are through the avoided-crossing momenta. (C) EDCs through Γ under linearly (red squares) and circularly (blue circles) polarized pump at $t = 0$ fs, as well as the EDC through Γ taken at $t = -500$ fs (black).

The surface Dirac Hamiltonian hybridized with circularly polarized light essentially results in a Chern insulator as originally proposed by Haldane (35). In his model, alternating current loops break TRS locally, and the total magnetization remains zero. Whereas such microscopic periodic current configuration is difficult to implement, the Hamiltonian of graphene per valley in the Haldane model is basically the same as that of a circularly polarized electromagnetic field coupled to a Dirac band (9), which is realized in our experiment. These observations suggest the existence of a photoinduced anomalous quantum Hall phase without Landau levels where topological magnetoelectric effects described by axion electrodynamics may exist (5).

The quantitative agreement between the measured gap sizes and the models based on Floquet theory shows the general applicability of our result to Dirac systems. These observations open up an avenue for optical controlling and switching topological orders and may provide a platform for realizing Floquet Majorana modes in these materials (1, 2).

References and Notes

1. M. Z. Hasan, C. L. Kane, *Rev. Mod. Phys.* **82**, 3045–3067 (2010).
2. X. L. Qi, S. C. Zhang, *Rev. Mod. Phys.* **83**, 1057–1110 (2011).
3. J. E. Moore, *Nature* **464**, 194–198 (2010).

4. C. Z. Chang *et al.*, *Science* **340**, 167–170 (2013).
5. X.-L. Qi, T. L. Hughes, S.-C. Zhang, *Phys. Rev. B* **78**, 195424 (2008).
6. Y. L. Chen *et al.*, *Science* **329**, 659–662 (2010).
7. S. Y. Xu *et al.*, *Nat. Phys.* **8**, 616–622 (2012).
8. H. Beidenkopf *et al.*, *Nat. Phys.* **7**, 939–943 (2011).
9. T. Kitagawa, T. Oka, A. Brataas, L. Fu, E. Demler, *Phys. Rev. B* **84**, 235108 (2011).
10. N. H. Lindner, G. Refael, V. Galitski, *Nat. Phys.* **7**, 490–495 (2011).
11. J. I. Inoue, A. Tanaka, *Phys. Rev. Lett.* **105**, 017401 (2010).
12. O. V. Yazyev, J. E. Moore, S. G. Louie, *Phys. Rev. Lett.* **105**, 266806 (2010).
13. C. Cohen-Tannoudji, J. Dupont-Roc, G. Grynberg, *Atom-Photon Interaction* (Wiley, Hoboken, NJ, 1992).
14. E. Goulielmakis *et al.*, *Nature* **466**, 739–743 (2010).
15. M. C. Rechtsman *et al.*, *Nature* **496**, 196–200 (2013).
16. F. M. Faisal, J. Z. Kaminski, *Phys. Rev. A* **56**, 748–762 (1997).
17. B. M. Fregoso, Y. H. Wang, N. Gedik, V. Galitski, <http://arxiv.org/abs/1305.4145> (2013).
18. V. M. Galitskii, S. P. Goreslavskii, V. F. Elesin, *Sov. Phys. JETP* **30**, 117 (1970).
19. C. Kittel, *Introduction to Solid State Physics* (Wiley, Hoboken, NJ, 2004).
20. H. Sambe, *Phys. Rev. A* **7**, 2203–2213 (1973).
21. M. Schultze *et al.*, *Nature* **493**, 75–78 (2013).
22. Q. T. Vu *et al.*, *Phys. Rev. Lett.* **92**, 217403 (2004).
23. S. Ghimire *et al.*, *Nat. Phys.* **7**, 138–141 (2011).
24. G. Saathoff, L. Miaja-Avila, M. Aeschlimann, M. M. Murnane, H. C. Kapteyn, *Phys. Rev. A* **77**, 022903 (2008).
25. L. Miaja-Avila *et al.*, *Phys. Rev. A* **79**, 030901 (2009).
26. J. A. Sobota *et al.*, *Phys. Rev. Lett.* **108**, 117403 (2012).

27. M. Hajlaoui *et al.*, *Nano Lett.* **12**, 3532–3536 (2012).
28. Y. H. Wang *et al.*, *Phys. Rev. Lett.* **109**, 127401 (2012).
29. Y. H. Wang *et al.*, *Phys. Rev. Lett.* **107**, 207602 (2011).
30. A. D. LaForge *et al.*, *Phys. Rev. B* **81**, 125120 (2010).
31. Materials and methods are available as supplementary materials on Science Online.
32. S. V. Syzranov, M. V. Fistul, K. B. Efetov, *Phys. Rev. B* **78**, 045407 (2008).
33. Y. Zhou, M. W. Wu, *Phys. Rev. B* **83**, 245436 (2011).
34. T. Oka, H. Aoki, *Phys. Rev. B* **79**, 081406 (2009).
35. F. D. Haldane, *Phys. Rev. Lett.* **61**, 2015–2018 (1988).

Acknowledgments: This work is supported by U.S. Department of Energy (DOE) award numbers DE-FG02-08ER46521 and DE-SC0006423 (data acquisition and analysis) and Army Research Office (ARO-DURIP) grant W911NF-09-1-0170 (electron spectrometer). H.S. and P.J.-H. have been supported by the DOE, Basic Energy Sciences Office, Division of Materials Sciences and Engineering, under award DE-SC0006418 (materials growth). This work made use of the Materials Research Science and Engineering Center Shared Experimental Facilities supported by NSF under award DMR-0819762. We are grateful for the stimulating discussions with L. Fu, T. Kitagawa, T. Oka, B. Fregoso, and V. Galitski.

Supplementary Materials

www.sciencemag.org/content/342/6157/453/suppl/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S4
References

30 April 2013; accepted 11 September 2013
10.1126/science.1239834

From Few to Many: Observing the Formation of a Fermi Sea One Atom at a Time

A. N. Wenz,^{1,2,*†} G. Zürn,^{1,2,†} S. Murmann,^{1,2} I. Brouzos,³ T. Lompe,^{1,2,4} S. Jochim^{1,2,4}

Knowing when a physical system has reached sufficient size for its macroscopic properties to be well described by many-body theory is difficult. We investigated the crossover from few- to many-body physics by studying quasi-one-dimensional systems of ultracold atoms consisting of a single impurity interacting with an increasing number of identical fermions. We measured the interaction energy of such a system as a function of the number of majority atoms for different strengths of the interparticle interaction. As we increased the number of majority atoms one by one, we observed fast convergence of the normalized interaction energy toward a many-body limit calculated for a single impurity immersed in a Fermi sea of majority particles.

The ability to connect the macroscopic properties of a many-body system to the microscopic physics of its individual constituent particles is one of the great achievements of physics. This connection is usually made using the assumption that the number of particles tends

to infinity. Then a transition from discrete to continuous variables can be made, which greatly simplifies the theoretical description of large systems. When does a system become large enough for this approximation to be valid? This is a difficult question to answer because most calculations based on a microscopic description become prohibitively complex before their predictions approach the many-body solution. Experimentally, this question has been studied in the context of helium droplets (1) and nuclear physics (2) by measuring the emergence of superfluidity for increasing system size. We addressed this question with the use of ultracold lithium atoms, which

have already been used to study few-particle systems with tunable interactions (3–5).

In our experiments, we control the system size on the single-particle level while maintaining full control over the interparticle interactions. We achieve this by deterministically preparing few-particle systems of ultracold ⁶Li atoms (6) whose interparticle interaction can be tuned using Feshbach resonances (7, 8). This allows us to explore the crossover from few- to many-body physics by studying the fermionic quantum impurity problem, where a single impurity atom interacts with a number of fermionic majority atoms. The majority atoms do not interact with each other because of the Pauli principle. In this system the impurity acts as a test particle, which we use to probe the majority component. The limit of a single majority particle (Fig. 1A) has been thoroughly

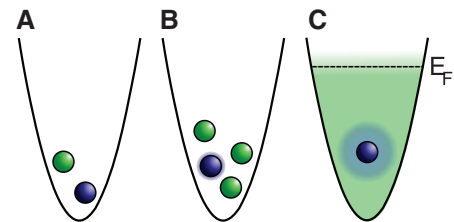


Fig. 1. From few to many. A single impurity (blue) interacting with one, few, and many fermions (green) in a harmonic trapping potential. In the many-body case, the majority component can be described as a Fermi sea with a Fermi energy E_F .

¹Physikalisches Institut, Ruprecht-Karls-Universität Heidelberg, 69210 Heidelberg, Germany. ²Max-Planck-Institut für Kernphysik, Saupfercheckweg 1, 69117 Heidelberg, Germany. ³Institut für Quanten-Informationsverarbeitung, Universität Ulm, 89069 Ulm, Germany. ⁴Extreme Matter Institute (EMMI), GSI Helmholtzzentrum für Schwerionenforschung, 64291 Darmstadt, Germany.

*Corresponding author. E-mail: wenz@physi.uni-heidelberg.de
†These authors contributed equally to this work.

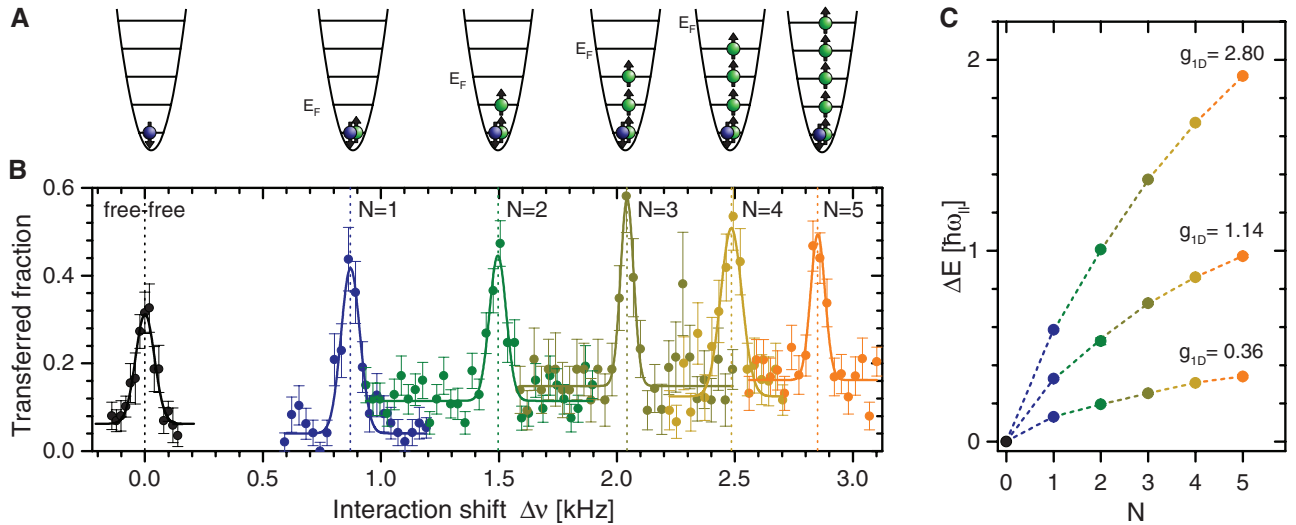


Fig. 2. Interaction energies. (A) Deterministically prepared noninteracting $(N + 1)$ -particle states. (B) Determined RF spectra for a single impurity interacting with different numbers of majority atoms with a coupling strength $g_{1D} = 2.80$ in units of $a_{\parallel}\hbar\omega_{\parallel}$. The transferred fraction denotes the average fraction of the minority atom transferred to a different hyperfine state. (C)

The interaction energies for different values of g_{1D} are determined by Gaussian fits to the experimental spectra [solid lines in (B)] and are plotted as a function of N . The errors of $\Delta E(N)$ are determined from the uncertainties of the fits to the experimental spectra (14) and are not visible because of their small size.

investigated both theoretically (9) and experimentally (3, 10). For a large number of majority atoms, the system can be described as a single impurity interacting with a Fermi sea (Fig. 1C). Similar physics has been experimentally explored by introducing a small fraction of impurity atoms into a large Fermi sea of ultracold atoms (11–13).

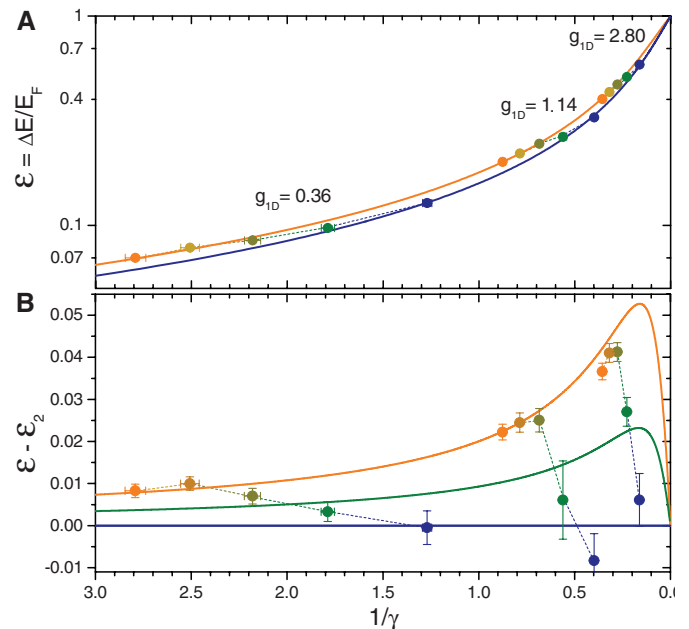
We prepare $(N + 1)$ -particle systems consisting of one impurity atom and N majority atoms in their N -particle ground state trapped in an elongated optical dipole potential (Fig. 2A) (6). We create these quasi-one-dimensional few-particle systems using ultracold fermionic ^6Li atoms in different hyperfine states, where we label the minority particle as $|\downarrow\rangle$ and the atoms of the majority component as $|\uparrow\rangle$. This system is very well described by the Hamiltonian

$$H = \sum_{i=0}^N \left(-\frac{\hbar^2}{2m} \frac{\partial^2}{\partial x_i^2} + \frac{1}{2} m \omega_{\parallel}^2 x_i^2 \right) + g_{1D} \sum_{i=1}^N \delta(x_i - x_0) \quad (1)$$

where x_0 is the position of a single impurity atom that interacts with N atoms of the majority component, which are located at the coordinates x_1 to x_N , through a contact interaction of strength g_{1D} ; $\hbar$ is Planck's constant divided by 2π ; m is the mass of a ^6Li atom; and $\omega_{\parallel}$ is the axial frequency of the harmonic trapping potential (14). This Hamiltonian can be solved analytically for the two limiting cases of a single majority particle ($N = 1$) (9) and an infinite number of majority particles in a homogeneous system ($\omega_{\parallel} \rightarrow 0$) (15).

To probe the system, we measure the interaction energy between the impurity and the majority component as a function of the number of

Fig. 3. Comparison with theory. (A) Normalized interaction energy as a function of the interaction parameter γ . The solid lines indicate the analytic predictions for $N = 1$ (9) (blue) and $N \rightarrow \infty$ (15) (orange). The colored dots represent the measured interaction energies for systems with $N = 1$ to 5 (colors according to Fig. 2), corrected for the effects of the finite aspect ratio of the trap. For the normalization of the interaction energies of the few-particle theories and the measurement, we have approximated the density by its peak density to compare it with the many-body theory developed for a homogeneous system (15). (B) Difference between the interaction energies and the theoretical prediction for a two-particle system ($N = 1$). The theoretical prediction for $N = 2$ (26) is shown as a green solid line. The measured interaction energies coincide with the two-particle prediction for $N = 1$; for $N \geq 4$ these energies approach the many-particle limit, which indicates the formation of a Fermi sea.



majority particles. We do this by changing the internal state of the single impurity atom from an initial hyperfine state $|\downarrow_i\rangle$ to a different hyperfine state $|\downarrow_f\rangle$ by means of a radio-frequency (RF) pulse (16, 17). If no majority atoms are present, the transition occurs at a frequency ν_0 corresponding to the hyperfine splitting between the initial and final states of the impurity atom. In the presence of N majority atoms, the inter-

actions between the impurity and the majority atoms lead to an interaction shift $\Delta\nu(N)$ of the transition frequency.

Using such RF spectroscopy measurements, we study systems with weak ($g_{1D} = 0.36$), intermediate ($g_{1D} = 1.14$), and strong ($g_{1D} = 2.80$) repulsive interactions, where g_{1D} is given in units of $a_{\parallel}\hbar\omega_{\parallel}$ and $a_{\parallel} = \sqrt{\hbar/(m\omega_{\parallel})}$ is the harmonic oscillator length. We perform these measure-

ments for systems containing one to five majority atoms. As an example, the resulting RF spectra for strong interaction are shown in Fig. 2B. Note that a trapped system has discrete eigenstates and thus all RF spectra consist of discrete transitions. Because the resolution of our RF spectroscopy is much higher than the level spacing $\hbar\omega_{\parallel}$ of our trap, we resolve these discrete transitions and therefore observe sharp transitions with symmetric line shapes. Hence, we can fit all these transitions with Gaussians and obtain the interaction energies $\Delta E(N) = \hbar\Delta\nu(N)$ for different coupling strengths g_{1D} (Fig. 2C).

The addition of each majority atom increases the number of particles with which the impurity atom can interact, and thus the interaction energy rises for increasing N . For weak interactions, it was shown that $\Delta E \propto \sqrt{N}$ (18), and hence ΔE diverges for $N \rightarrow \infty$. Therefore, we rescale the interaction energy by the natural energy scale of the system, the Fermi energy of the majority atoms E_F , to obtain the dimensionless interaction energy $\epsilon = \Delta E/E_F$. In a trapping potential, the Fermi energy is determined by the energy of the lowest single-particle level not occupied by a majority atom. Because we consider only the gain in interaction energy, we neglect the zero-point motion, which means that $E_F = N\hbar\omega_{\parallel}$ for the case of a harmonic trap. For our optical trap, which is slightly anharmonic, we determine the Fermi energy for each N by precise measurements of the level spacings and Wentzel-Kramers-Brillouin calculations (10, 14).

To compensate for the change in density caused by an increase in the number of particles, we rescale g_{1D} with the line density of the majority atoms. For the trapped systems, we approximate the line density by its peak density given by the Fermi wave vector $k_F = \sqrt{2mE_F}/\hbar$, which has been shown to be very accurate even for low particle numbers (18). As a result, we obtain a dimensionless interaction parameter $\gamma = (\pi m/\hbar^2)g_{1D}/k_F$ (19).

To investigate how the measured interaction energy of our system approaches the many-body limit, we compare our data with theoretical predictions for the two limiting cases of one and an infinite number of majority particles. The normalized interaction energy $\epsilon_2 = \Delta E_2/E_F$ of the two-particle system consisting of an impurity atom interacting with only one majority atom (9) is shown as a blue line in Fig. 3. In the many-body limit where a single impurity is immersed in a Fermi sea of an infinite number of majority atoms, an explicit expression for the interaction energy in a homogeneous one-dimensional system has been analytically calculated (15) to be

$$\epsilon_{\infty} = \frac{\Delta E_{\infty}}{E_F} = \frac{\gamma}{\pi^2} \left[1 - \frac{\gamma}{4} + \left(\frac{\gamma}{2\pi} + \frac{2\pi}{\gamma} \right) \tan^{-1} \left(\frac{\gamma}{2\pi} \right) \right] \quad (2)$$

This interaction energy for the many-body case is shown in Fig. 3 as an orange line.

The two theories coincide for the limits of vanishing and diverging interactions. For $\gamma = 0$, the interaction energy is zero. For $\gamma \rightarrow \infty$, one reaches the limit of fermionization where the energy of the impurity atom interacting with N majority fermions exactly matches the energy of $N + 1$ noninteracting identical fermions, and therefore the gain in interaction energy is equal to the Fermi energy E_F (10, 15, 20–25). Between these limits, the many-body solution consistently has a slightly larger normalized interaction energy than the two-body solution.

To compare our data to these strictly one-dimensional theories, we must consider the fact that our quasi-one-dimensional trapping potential has a finite aspect ratio of about 10:1. We estimate this effect on the interaction energy using numerical calculations (26–28) and find that the largest corrections occur for intermediate and strong interactions, and is on the order of 2% of the interaction energy (14). The corrected data are plotted in Fig. 3A as a function of the dimensionless interaction parameter γ ; the different colors indicate the number of majority atoms. The measured interaction energy quickly approaches the many-body limit as the number of majority particles increases. To show this in more detail, we subtract the interaction energy given by the two-particle theory ϵ_2 from the total interaction energy (Fig. 3B).

For the two-particle system, where the impurity interacts with only one majority atom ($N = 1$), we find excellent agreement between the experimental data (blue dots) and its analytical prediction (blue line). For two or more majority atoms ($N \geq 2$), there is no analytic prediction for the interaction energy, but recent state-of-the-art numerical calculations exist (18, 26, 29). We compare our experimental results for $N = 2$ (green dots) to the calculations of (26) (green line) and find good agreement. Note that adding only one atom to the two-particle system leads to an increase of the normalized interaction energy, which is already about half of the energy gain expected for the $N \rightarrow \infty$ limit.

For more than two majority particles, one observes a quick convergence of the experimentally determined interaction energy toward the many-body limit described by the analytical solution in (15). For strong interactions, the data points follow the shape of the many-body theory but have a slight offset. Possible reasons for this deviation might be the peak density approximation and the uncertainties in our corrections for the anharmonicity and the finite aspect ratio, which are largest for strong interactions. For weak and intermediate interactions, the determined interaction energy agrees with the many-body theory within the experimental errors for as few as four majority atoms. This fast convergence of the normalized interaction energy shows that even for very few particles, the many-body theory describes essential aspects of our system.

Further insight into the one-dimensional quantum impurity problem can be gained by considering

our findings in terms of polaron-like behavior. In general, a polaron is a quasiparticle that consists of an impurity coherently dressed by particle-hole excitations of the Fermi sea (30–32). In a homogeneous one-dimensional system, polaronic quasiparticles are not expected to exist, but polaron-like behavior for weak interactions can still be observed (33). To understand how this behavior emerges for growing N requires further measurements of the effective mass, which can be obtained by investigating the excitation spectrum of the system.

For strong interactions, the polaron-like description breaks down in a one-dimensional system, and for $g_{1D} \rightarrow \infty$ one approaches the limit of fermionization. Then the system's energy reaches that of a system of $(N + 1)$ noninteracting identical fermions (34). By crossing this point of fermionization, one can reach a metastable state where the interaction energy is larger than the Fermi energy (10), which might lead to the appearance of nontrivial spin correlations (25, 29, 35). For spin-balanced systems with attractive interactions, our setup could be used to study the emergence of pairing (36) and superfluidity in a fermionic few-particle system with tunable interactions and full control over the system's quantum state.

References and Notes

1. S. Grebenev, J. P. Toennies, A. F. Vilesov, *Science* **279**, 2083–2086 (1998).
2. A. Migdal, *Nucl. Phys.* **13**, 655–674 (1959).
3. C. Ospelkaus *et al.*, *Phys. Rev. Lett.* **97**, 120402 (2006).
4. S. Will *et al.*, *Nature* **465**, 197–201 (2010).
5. S. Will, T. Best, S. Braun, U. Schneider, I. Bloch, *Phys. Rev. Lett.* **106**, 115305 (2011).
6. F. Serwane *et al.*, *Science* **332**, 336–338 (2011).
7. C. Chin, R. Grimm, P. Julienne, E. Tiesinga, *Rev. Mod. Phys.* **82**, 1225–1286 (2010).
8. T. Bergeman, M. G. Moore, M. Olshanii, *Phys. Rev. Lett.* **91**, 163201 (2003).
9. T. Busch, B.-G. Englert, K. Rzaewski, M. Wilkens, *Found. Phys.* **28**, 549–559 (1998).
10. G. Zürn *et al.*, *Phys. Rev. Lett.* **108**, 075303 (2012).
11. A. Schirotzek, C.-H. Wu, A. Sommer, M. W. Zwierlein, *Phys. Rev. Lett.* **102**, 230402 (2009).
12. M. Koschorreck *et al.*, *Nature* **485**, 619–622 (2012).
13. C. Kohstall *et al.*, *Nature* **485**, 615–618 (2012).
14. See supplementary materials on Science Online.
15. J. B. McGuire, *J. Math. Phys.* **6**, 432 (1965).
16. C. Chin, P. S. Julienne, *Phys. Rev. A* **71**, 012713 (2005).
17. G. Zürn *et al.*, *Phys. Rev. Lett.* **110**, 135301 (2013).
18. G. E. Astrakharchik, I. Brouzos, *Phys. Rev. A* **88**, 021602 (2013).
19. The dimensionless interaction parameter is also known as the Lieb-Liniger parameter.
20. M. Girardeau, *J. Math. Phys.* **1**, 516 (1960).
21. T. Kinoshita, T. Wenger, D. S. Weiss, *Science* **305**, 1125–1128 (2004).
22. E. Haller *et al.*, *Science* **325**, 1224–1227 (2009).
23. M. D. Girardeau, *Phys. Rev. A* **82**, 011607 (2010).
24. I. Brouzos, P. Schmelcher, *Phys. Rev. A* **87**, 023605 (2013).
25. E. J. Lindgren, J. Rotureau, C. Forssén, A. G. Volosniev, N. T. Zinner, <http://arxiv.org/abs/1304.2992> (2013).
26. S. E. Gharashi, K. M. Daily, D. Blume, *Phys. Rev. A* **86**, 042702 (2012).
27. Z. Idziaszek, T. Calarco, *Phys. Rev. A* **71**, 050701 (2005).
28. G. Zürn, thesis, Ruprecht-Karls-Universität Heidelberg (2012).

29. P. O. Bugnion, G. J. Conduit, *Phys. Rev. A* **87**, 060502 (2013).
30. N. Prokof'ev, B. Svistunov, *Phys. Rev. B* **77**, 020408 (2008).
31. N. V. Prokof'ev, B. V. Svistunov, *Phys. Rev. B* **77**, 125101 (2008).
32. S. Giraud, R. Combescot, *Phys. Rev. A* **79**, 043615 (2009).
33. X.-W. Guan, M. T. Batchelor, C. Lee, <http://arxiv.org/abs/1301.6446> (2013).
34. Because the Fermi energy is also the only remaining energy scale in a 3D system, we expect a similar convergence to a universal value determined by the Fermi energy times a numerical constant.
35. S. E. Gharashi, D. Blume, *Phys. Rev. Lett.* **111**, 045302 (2013).
36. G. Zürn, A. N. Wenz, S. Murmann, T. Lompe, S. Jochim, <http://arxiv.org/abs/1307.5153> (2013).

Acknowledgments: We thank D. Blume and S. E. Gharashi for inspiring discussions and for providing the results of their numerical calculations, and R. Schmidt, X. Guan, and J. McGuire for very helpful discussions. I.B. thanks G. E. Astrakharchik for helpful discussions. Supported by the International Max Planck Research School for Quantum Dynamics (A.N.W. and G.Z.), the European Commission-funded Future and Emerging Technologies project Quantum Interferometry with Bose-Einstein

Condensates (I.B.), European Research Council starting grant 279697, the Helmholtz Alliance (HA216-EMMI), and the Heidelberg Center for Quantum Dynamics.

Supplementary Materials

www.sciencemag.org/content/342/6157/457/suppl/DC1
Materials and Methods
Figs. S1 to S6
Tables S1 to S3
References and Notes (37, 38)

14 May 2013; accepted 13 September 2013
10.1126/science.1240516

Stabilizing Liquid Drops in Nonequilibrium Shapes by the Interfacial Jamming of Nanoparticles

Mengmeng Cui,¹ Todd Emrick,¹ Thomas P. Russell^{1,2,*}

Nanoparticles assemble at the interface between two fluids into disordered, liquid-like arrays where the nanoparticles can diffuse laterally at the interface. Using nanoparticles dispersed in water and amine end-capped polymers in oil, nanoparticle surfactants are generated in situ at the interface overcoming the inherent weak forces governing the interfacial adsorption of nanoparticles. When the shape of the liquid domain is deformed by an external field, the surface area increases and more nanoparticles adsorb to the interface. Upon releasing the field, the interfacial area decreases, jamming the nanoparticle surfactants and arresting further shape change. The jammed nanoparticles remain disordered and liquid-like, enabling multiple, consecutive deformation and jamming events. Further stabilization is realized by replacing monofunctional ligands with difunctional versions that cross-link the assemblies. The ability to generate and stabilize liquids with a prescribed shape poses opportunities for reactive liquid systems, packaging, delivery, and storage.

Imagine being able to control the shape of one liquid in another liquid where a protective layer (1) between the liquids was produced in situ and the shapes of the liquids are locked-in. This would enable the design of interpenetrating, two-phased systems (2) where the morphologies and flow characteristics are tailored, the interfacial area can be manipulated and decorated with functional groups (3–5), or three-dimensional (3D) nano- or microfluidic devices can be produced in a simple manner. The interfacial jamming of particles is a promising route to this goal (6, 7). The jamming of colloidal particles at interfaces has been discussed for the fusion (8, 9) and coalescence of liquid drops (10) and the generation of “bijels” (11, 12), pointing to the importance of jamming to freeze-in, or solidify, otherwise liquid structures. From size considerations alone, nanoparticles extend the size scale of accessible structures to a much smaller dimension. Yet, confining the colloidal particles to the interface so as to achieve long-term stability of the assemblies and drop shape requires modifying the surface chem-

istry of the particles to achieve neutral wetting conditions (11–14). This criterion is even more important for nanoparticles, where the reduction

in the interfacial energy of each nanoparticle is close to the thermal energy (7, 15, 16). Previous studies demonstrated that nonequilibrium shapes could be produced (17–21), but tailoring and preserving the shape or reducing the size scale of the structures was not discussed. Here, we use a uniform electric field to control the shape of fluid drops that are suspended in a second fluid. This deforms the drop (Fig. 1) by an amount that depends on the strength of the applied field and, inversely, the interfacial energy.

In this study, nanoparticles, dispersed in a water drop that is suspended in an oil, self-assemble at the interface to reduce the interfacial energy, forming a monolayer of nanoparticles at the oil-water interface that pack in a liquid-like manner (22–24). The decrease in the interfacial energy per nanoparticle is enhanced by dissolving an end-functionalized polymer in the oil phase that interacts with the nanoparticles, forming nanoparticle surfactants. The assembly of nanoparticle surfactants saturates the interface to maximize the reduction in interfacial energy and form a disordered, jammed assembly. Upon application of an electric field, the drop deforms, and the surface area (not the volume) of the drop increases and

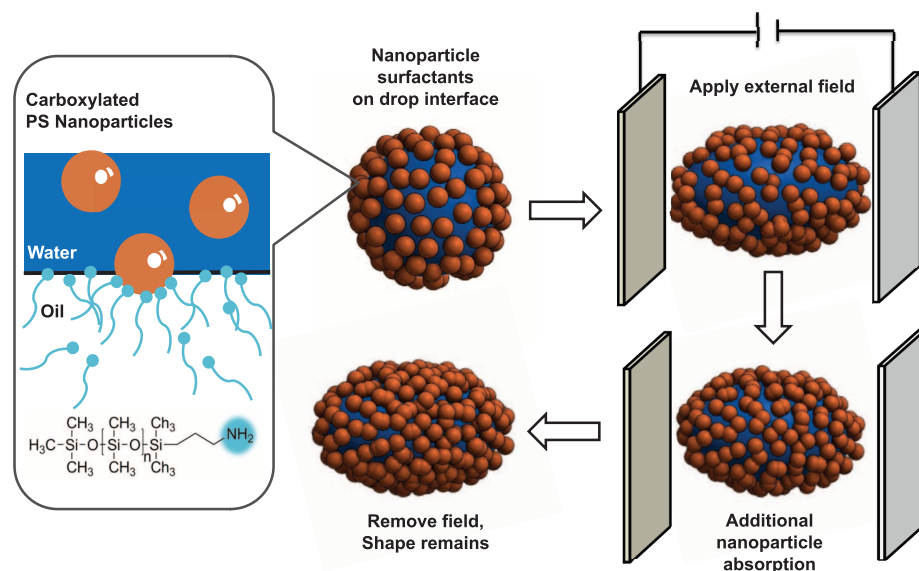


Fig. 1. Schematic representation of the deformation of a spherical drop, cladded with nanoparticle surfactants, by an electric field, into an ellipsoid whose shape is maintained after the removal of the field by the interfacial jamming of the nanoparticle surfactants.

¹Polymer Science and Engineering Department, University of Massachusetts, 120 Governors Drive, Amherst, MA 01003, USA.

²WPI-Advanced Institute for Materials Research, Tohoku University, 2-1-1 Katahira, Aoba, Sendai 980-8577, Japan.

*Corresponding author. E-mail: russell@mail.pse.umass.edu

unjams the nanoparticle surfactant assembly. The increased surface area allows the formation and assembly of additional nanoparticle surfactants at the interface. Upon removal of the field, the drop attempts to return to its lowest energy (spherical) shape to minimize the interfacial area. However, the assembled layer of nanoparticle surfactants jams, arresting further shape changes in the drop and kinetically trapping the drop into a shape far removed from equilibrium.

When an aqueous dispersion of carboxylated polystyrene nanoparticles was placed in a mixture of oils, consisting of 95 volume percent (volume %) of a higher viscosity ($\eta = 60,000$ cSt) silicone oil and 5 volume % of a lower viscosity silicone oil ($\eta = 50$ cSt) with an amine end-group [an amine-terminated polydimethylsiloxane (PDMS)], nanoparticle surfactants formed as a result of carboxylate-amine interactions and assembled at the interface, reducing the interfacial energy. The number of polymers interacting with the nanoparticle is limited by the minimum in the energy, balancing the solubilization of the surfactant in each fluid and the reduction in the interfacial energy. Consequently, the system is self-regulating.

As shown in Fig. 2A, the spherical water drop is stretched into a prolate ellipsoid under the influ-

ence of the applied electric field (4.6 kV/cm), increasing the surface area from 40.7 to 193.2 mm². Over time, the drop stretches further, increasing the major axis of the ellipsoid, while the minor axis decreases, up to a point where the increase in surface area—i.e., interfacial energy—balances the applied force of the electric field. When the field is shut off, the deformation of the drop decreases slightly to a surface area of 189.0 mm², at which point the nanoparticle surfactants are jammed at the interface, arresting further relaxation and kinetically trapping the drop in a non-equilibrium, nonspherical shape (fig. S1 and movie S1). Figure 1B shows the exceptional stability of the deformed water drop after removal of the electric field. Even after 1 month, the drop retained the highly deformed shape. Any shape changes are attributable to solvent evaporation (movie S2). It should be noted that if the volume of the drop decreases, either intentionally or due to evaporation, the jammed nanoparticle surfactant assembly will wrinkle and buckle, depending on the amount of volume loss, in a manner resembling raisins or other dried fruits (see fig. S2). However, without volume contraction, no wrinkling occurs.

The leaky dielectric model introduced by Taylor (25–27) was used to relate the deforma-

tion D of a spherical liquid drop to the strength of the applied electric field E

$$D = \frac{9\epsilon_0\epsilon_d\Phi aE^2}{16\gamma(2+R)^2}$$

$$\Phi \equiv S(R^2 + 1) - 2 + 3(SR - 1)\frac{2M + 3}{5M + 5}$$

where $D = (d_1 - d_2)/(d_1 + d_2)$, d_1 and d_2 are the major and minor axes of the ellipsoid formed, a is the radius of the initial drop, γ is the interfacial tension, ϵ_0 is the permittivity of free space, and ϵ_d is the permittivity of the liquid inside the drop. S is the ratio of the dielectric permittivities of the drop to the surrounding liquid, R is the ratio of the respective conductivities, and M is the ratio of the respective viscosities. Since the conductivity of water is much larger than that of silicone oil, $R \sim 10^6$, to a very good approximation

$$D \approx \frac{aE^2}{\gamma}$$

and the deformation of the drop should vary linearly with E^2 and have a slope of a/γ . If the drop is highly conductive (as is the case in our experiments), the electrostatic pressure acting on the interface to deform the drop is countered by energetic gain arising from the increased interfacial area (28–30). So, the deformation of the drop could also be explained by a balance of electric and mechanical forces, yielding the same result. In the limit of small deformation, as shown in Fig. 2D, we can determine the interfacial tension.

The time and E^2 dependence of D are shown in Fig. 2, C and D, respectively. In Fig. 2C, a sequential deformation of the drop, with the field turned on then off, reaches an asymptotic value dictated by the field strength and the number of nanoparticle surfactants formed at the interface. In Fig. 2D, with only nanoparticles in the water phase, the drop deformation is identical to that seen with pure water in oil. With only functionalized PDMS in the oil phase, the rate at which D changed with E^2 was 2.1 times as high, indicating that the functionalized PDMS acts like a surfactant, decreasing γ . However, when the field was removed, the drop returned to its spherical shape. When the nanoparticles and functionalized PDMS are in the water and oil phases, respectively, the rate at which D increased with E^2 was 2.6 times higher than the pure fluids, indicating a further small reduction in γ due to the in situ formation of the nanoparticle surfactants. With the assumption that the conductivity, viscosity, and permittivity ratios are constant, we can determine γ values, because γ between water and silicone oil is 25 mN/m. γ for pure water with the silicone oil mixture containing amino end-capped silicone oil is 12.14 mN/m, and γ for water with the nanoparticles in the silicone oil mixture containing amine-capped silicone oil is 9.58 mN/m. Similar results were also found for the toluene/water system (fig. S3). These results provide insight

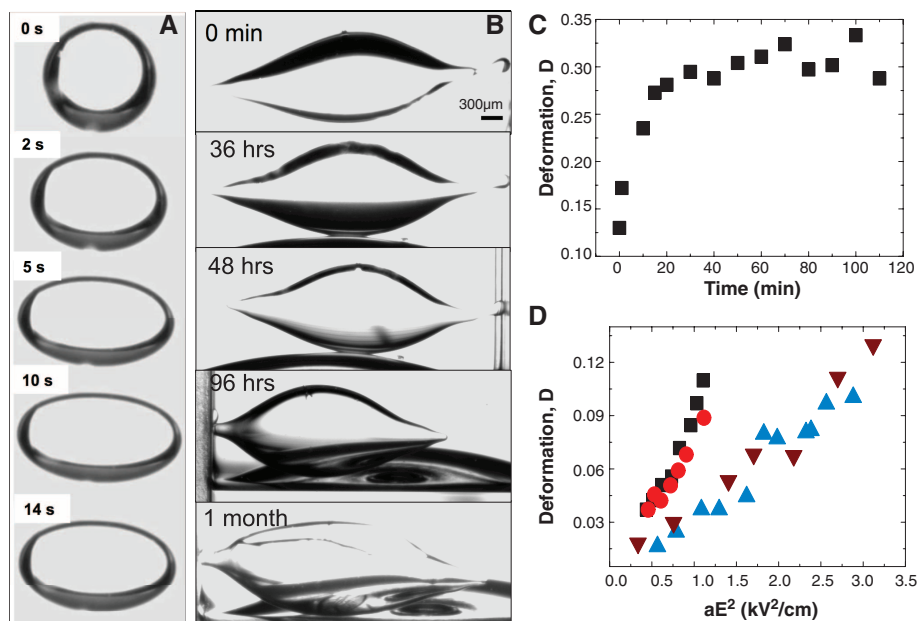


Fig. 2. The deformation and stability of drops clad with nanoparticle surfactants. (A) A time series showing the deformation of a water drop, containing 1% (by weight) dispersion of 15 nm carboxylated polystyrene nanoparticles, suspended in a silicone oil containing PDMS, end-functionalized with an amine, under a 4.6-kV/cm electric field. The diameter of the original drop is 1.8 mm. (B) A time series of a drop, clad with nanoparticle surfactants, that has been deformed in an electric field and the field has been shut off. (C) The deformation D of the water drop containing polystyrene nanoparticles in a silicone oil containing amine end-capped PDMS. D asymptotes to a well-defined value defined by the force exerted by the electric field and the reduction in interfacial energy. (D) The linear relationship between the D and aE^2 under various conditions. The maroon triangles represent pure water in pure, nonfunctional silicone oil; the blue triangles are results from polystyrene nanoparticles in water dispersed in a nonfunctional silicone oil; the red circles represent pure water in mixed silicone oils, one of which is an amine end-capped PDMS (5%); the black circles are results for a drop of an aqueous dispersion of polystyrene nanoparticles (1%) in mixed silicone oils, one of which is an amine end-capped PDMS (5%).

into the mechanism of the formation of the nanoparticle surfactants. It is apparent that the nanoparticles alone do not exhibit strong interfacial activity, whereas the amine end-capped silicone oil does. Thus, it is reasonable to conclude that the amine-capped silicone oil initially assembles

at the interface, and the nanoparticles then diffuse to the interface and interact with the amine end-groups on the silicone oil to form the nanoparticle surfactant.

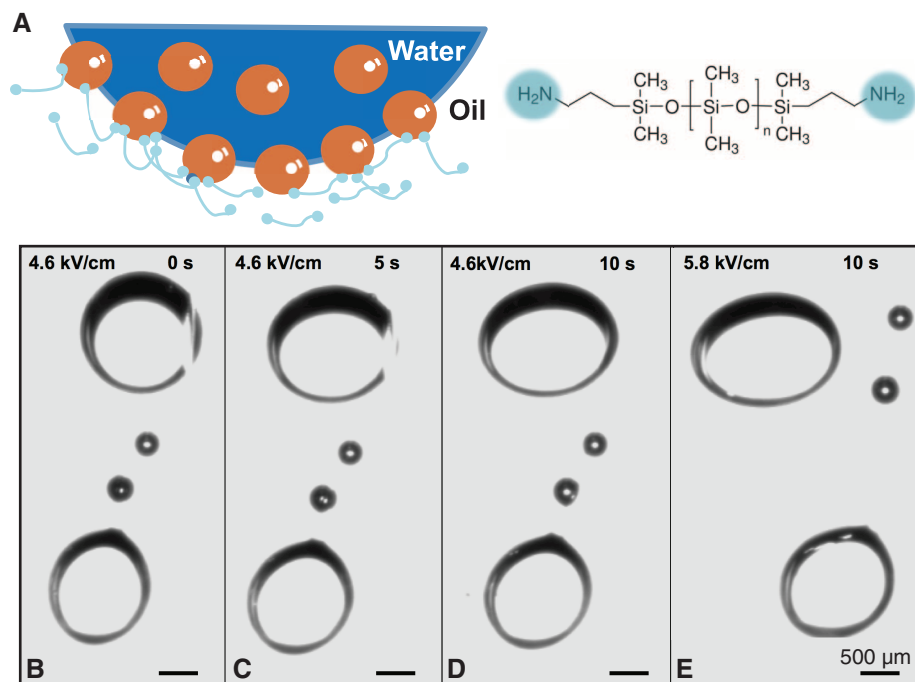
The unjamming of the nanoparticle surfactant assemblies opens opportunities to control the shape

of the fluid drops and stabilize highly nonequilibrium shapes. The nanoparticle surfactant assembly does not show cracking when the drop is deformed, indicative of its jammed state, as opposed to being glassy or ordered. By multiple jamming and unjamming steps, where the direction of the applied field is changed, unusual shapes are produced that are stable for at least 1 month. Shown in Fig. 3 is a water drop containing carboxylated polystyrene nanoparticles in an oil containing amine end-capped PDMS that is sequentially subjected to electric fields in the orthogonal directions. Applying the field in the x direction deforms the drop into an ellipsoid, with the major axis in the direction of the applied field (Fig. 3B). Then the field was reapplied, deforming the drop further in the x direction (Fig. 3C and movie S3), yielding tubular structures extending from the drop. An electric field was then applied orthogonal to this—i.e., in the y direction—which resulted in a further deformation of the drop, producing a similar type of tubular structure in the y direction (Fig. 3D, fig. S4A, and movie S4). These results demonstrate that the unjamming of the nanoparticle surfactant assembly initially occurs locally, enabling this independent deformation in different directions. Figure 3E shows a liquid drop that was treated after another electric field was placed along the y direction similarly, leaving a fish-shaped drop that remained in this state for 1 month before the experiment was terminated (fig. S4B and movie S5).

The generality of drop deformation is shown in Fig. 3F, where a stirring rod was placed within a drop of the aqueous dispersion of nanoparticles suspended in the mixed silicone oil. The rod was then placed in the aqueous phase and dragged through the oil, resulting in an aqueous tube clad with the nanoparticle surfactants that traced the path of the rod. When the system was stirred, the

Fig. 3. The deformation of a nanoparticle surfactant-stabilized water drop by the sequential application of an electric field in orthogonal directions. The diameter of the water drop is 1.5 mm. (A) The drop is deformed into an ellipsoid with an electric field (4.5 kV/cm). (B) The drop was further deformed in the same direction with a larger electric field (5.4 kV/cm). (C) A field of 4.5 kV/cm was then applied orthogonal to the original field direction—i.e., in the y direction—producing protrusions in the y direction on the already deformed drop (D). By using another field of 5.4 kV/cm applied along the y direction, unusual shapes, like the fish-like drop shown (E), could be produced and stabilized indefinitely. Different morphologies are shown by using a stirring rod to deform the drop. (F) The drop after a minimal amount of stirring, where aqueous tubes, clad with nanoparticle surfactants, traced the path of the rod. (G) A rapid, longer stirring produced a “glass wool”-type structure composed of small-diameter aqueous tubes clad with nanoparticle surfactants.

Fig. 4. A route to stabilize nanoparticle surfactant assemblies with difunctional PDMS. (A) A schematic showing the bridging of the difunctionalized PDMS to adjacent nanoparticles, cross-linking the nanoparticle assembly at the interface. The deformation of two drops containing an aqueous dispersion of carboxylated polystyrene nanoparticles in a silicone oil containing difunctional PDMS with an amine group on each end is shown in the lower frames (B to E). The drop on the top is in contact with the oil mixture for 30 s, whereas the lower drop is in contact with the mixed oil for 2 min. The initial drops are shown in (B). An electric field (4.6 kV/cm) is applied for 5 s and the upper drop deforms, while the lower drop retains its shape (C). After 10 s in the electric field, the upper drop deforms further and the lower drop retains its shape (D). The electric field is increased to 5.8 kV/cm, the upper drop deforms further, and only a very small change is seen in the lower drop (E).



aqueous tubes clad with the nanoparticle surfactants were smaller in diameter and exceptionally long, reminiscent of glass wool (Fig. 3G) except that the structures are fully liquid. The 3D nature of the morphology, the continuity of the tubular structures, and the cladding of the interfaces by the nanoparticle surfactant are evident. Thus, simply by stirring, a bicontinuous jammed system—a “bijel” type of morphology—is achieved without the need to modify and tune the surface chemistry of the nanoparticle to achieve neutral wetting, which is necessary to confine the nanoparticles to the liquid/liquid interface and impart long-term stability to the morphology (13, 14). The carboxylate-amine interactions between the nanoparticles and functionalized silicone oil are self-regulating, maximizing the reduction in the interfacial energy, overcoming thermal energies and stabilizing the nanoparticle surfactants at the interface.

When the monofunctional end-capped silicone oil was replaced with a difunctional PDMS, capped on both ends with primary amines, the nanoparticle surfactant assemblies were stabilized even further, as the PDMS chains bridge adjacent nanoparticles, effectively cross-linking the jammed nanoparticle assembly (Fig. 4A). Shown in Fig. 4, B to E, are two drops of water with polystyrene nanoparticles that were suspended in silicone oil containing the difunctional PDMS. The length of time that the drop at the bottom was in contact with the silicone oil is 2 min, whereas the contact time of the upper drop is 30 s. A 4.6-kV/cm electric field is applied, and the drop at the bottom does not deform, whereas the drop at the top deforms. Even though the interfacial energy of the drop at the bottom is lower, due to the increase in the number of nanoparticle surfactants formed at the interface, the cross-linking of the nanoparticle surfactants is greater, preventing the deformation of the drop (movies S6 and S7).

The very strong resistance of the drop to deformation demonstrates an alternate route by which drops of different shapes can be stabilized.

We have demonstrated a simple route to produce and stabilize fluid drops having shapes far removed from their equilibrium spherical shape, using the in situ formation of nanoparticle surfactants. The increased interfacial activity of the nanoparticle surfactants stabilized the assemblies against desorption, allowing them to jam at the interface, arresting change in the drop shape, and imparting long-term stability to drops with unusual shapes. The sequential application of external fields in different directions leads to local unjamming and jamming of the assemblies, enabling the shape of the drop to be tailored into a wide range of unusual shapes. We are currently investigating the possibility of stabilizing asymmetric shapes to determine the importance on the jamming process. Both electric and shear fields were used to deform the drops, although other fields, like magnetic and ultrasonic fields, are also being investigated. Cross-linking the nanoparticle surfactant assemblies at the interface is shown to increase the stability of the drop shape. These stabilized assemblies provide easy routes for encapsulation, bicontinuous flow (microfluidic devices) or separations media, delivery vehicles, and reaction platforms.

References and Notes

1. R. Hong *et al.*, *J. Am. Chem. Soc.* **128**, 1078–1079 (2006).
2. Y. Q. Shi, F. Li, Y. W. Chen, *New J. Chem.* **37**, 236–244 (2013).
3. K. Bramhaiah, N. S. John, *RSC Adv.* **3**, 7765 (2013).
4. D. Lee, D. A. Weitz, *Adv. Mater.* **20**, 3498–3503 (2008).
5. C. N. R. Rao, K. P. Kalyanikutty, *Acc. Chem. Res.* **41**, 489–499 (2008).
6. A. D. Dinsmore *et al.*, *Science* **298**, 1006–1009 (2002).
7. Y. Lin, H. Skaff, T. Emrick, A. D. Dinsmore, T. P. Russell, *Science* **299**, 226–229 (2003).
8. A. B. Subramaniam, M. Abkarian, L. Mahadevan, H. A. Stone, *Nature* **438**, 930 (2005).

9. M. Abkarian *et al.*, *Phys. Rev. Lett.* **99**, 188301 (2007).
10. A. B. Pawar, M. Caggioni, R. Ergun, R. W. Hartel, P. T. Spicer, *Soft Matter* **7**, 7710 (2011).
11. K. Stratford, R. Adhikari, I. Pagonabarraga, J. C. Desplat, M. E. Cates, *Science* **309**, 2198–2201 (2005).
12. E. M. Herzog, K. A. White, A. B. Schofield, W. C. K. Poon, P. S. Clegg, *Nat. Mater.* **6**, 966–971 (2007).
13. B. P. Binks, S. O. Lumsdon, *Langmuir* **16**, 8622–8631 (2000).
14. B. P. Binks, T. S. Horozov, *Colloidal Particles at Liquid Interfaces* (Cambridge Univ. Press, Cambridge, 2006).
15. L. Li *et al.*, *Nano Lett.* **11**, 1997–2003 (2011).
16. C. Zeng, F. Brau, B. Davidovitch, A. D. Dinsmore, *Soft Matter* **8**, 8582 (2012).
17. P. S. Clegg *et al.*, *J. Phys. Condens. Matter* **17**, S3433 (2005).
18. P. S. Clegg *et al.*, *Langmuir* **23**, 5984–5994 (2007).
19. H. L. Cheng, S. S. Velankar, *Langmuir* **25**, 4412–4420 (2009).
20. J. W. Tavares, J. H. J. Thijssen, A. B. Schofield, P. S. Clegg, *Adv. Funct. Mater.* **21**, 2020–2027 (2011).
21. S. A. F. Bon, S. D. Mookhoek, P. J. Colver, H. R. Fischer, S. van der Zwaag, *Eur. Polym. J.* **43**, 4839–4842 (2007).
22. D. Orsi, L. Cristofolini, G. Baldi, A. Madsen, *Phys. Rev. Lett.* **108**, 105701 (2012).
23. Y. Lin *et al.*, *Langmuir* **21**, 191–194 (2005).
24. A. J. Liu, S. R. Nagel, *Nature* **396**, 21–22 (1998).
25. G. Taylor, *Proc. R. Soc. London A Math. Phys. Sci.* **280**, 383–397 (1964).
26. O. Vrizika, D. A. Saville, *J. Fluid Mech.* **239**, 1 (1992).
27. D. A. Saville, *Annu. Rev. Fluid Mech.* **29**, 27–64 (1997).
28. H. A. Stone, J. R. Lister, M. P. Brenner, *Proc. R. Soc. London A* **455**, 329–347 (1999).
29. J. W. Ha, S. M. Yang, *J. Fluid Mech.* **405**, 131–156 (2000).
30. J. Q. Feng, T. C. Scott, *J. Fluid Mech.* **311**, 289 (1996).

Acknowledgments: This work was supported by the U.S. Department of Energy Office of Basic Energy Science through contract DE-FG02-04ER46126. There are no conflicts of interest.

Supplementary Materials

www.sciencemag.org/content/342/6157/460/suppl/DC1
Materials and Methods
Figs. S1 to S4
Movies S1 to S7

8 July 2013; accepted 25 September 2013
10.1126/science.1242852

Mass-Independent Oxygen Isotopic Partitioning During Gas-Phase SiO₂ Formation

Subrata Chakraborty,* Petia Yanchulova, Mark H. Thieme

Meteorites contain a wide range of oxygen isotopic compositions that are interpreted as heterogeneity in solar nebula. The anomalous oxygen isotopic compositions of refractory mineral phases may reflect a chemical fractionation process in the nebula, but there are no experiments to demonstrate this isotope effect during particle formation through gas-phase reactions. We report experimental results of gas-to-particle conversion during oxidation of silicon monoxide that define a mass-independent line (slope one) in oxygen three-isotope space of ¹⁸O/¹⁶O versus ¹⁷O/¹⁶O. This mass-independent chemical reaction is a potentially initiating step in nebular meteorite formation, which would be capable of producing silicate reservoirs with anomalous oxygen isotopic compositions.

The oxygen isotopic composition of high-temperature mineral phases in the first condensates in the protoplanetary disk,

calcium-aluminum-rich inclusions (CAIs), are distributed along a slope one line in an oxygen three-isotope plot (¹⁸O/¹⁶O versus ¹⁷O/¹⁶O) with a large

and equal depletion in ¹⁷O and ¹⁸O [~50 per mil (‰) with respect to the terrestrial composition] (1). Most chondrules (glassy globular condensates) formed shortly (<1 million years) after CAIs (2) display an oxygen isotopic distribution along an approximate slope 1 line, with about equal ¹⁷O and ¹⁸O enrichments over CAIs (3, 4). The oxygen isotopic distributions are notable because of their departure from the normal terrestrial mass-dependent (MD) fractionation line observed for equilibrium and kinetic fractionation processes of slope one-half (5, 6). Defining the source of the anomalous isotopic distribution of oxygen is critical in the elucidation of the overall formation and evolutionary events in the early solar system.

After the failure to find meteoritic supernova debris signatures, the initially proposed nuclear

Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla, CA 92093-0356, USA.

*Corresponding author. E-mail: subrata@ucsd.edu

theory (1) was largely abandoned, and chemical, particularly photochemical, theories took precedence as the favored explanation for the observed oxygen isotopic distribution. Photochemical isotopic self-shielding of CO (7) has been proposed on the basis of the predissociative nature (isotopologue-specific absorption lines) in the vacuum ultraviolet spectral region of CO, a common phenomenon in molecular clouds (8). Ozone formation was the first demonstration of a mass-independent (MI) fractionation process in a chemical reaction (9). Since this discovery, the potential for producing a CAI-like isotopic compositional trend in a gas-phase symmetry-driven recombination reaction has been suggested (9–11). Marcus (12) introduced a symmetry-based grain-surface assisted theoretical treatment of recombination of adsorbed species, e.g., O and SiO, to explain the compositions of CAIs. At present, there are no experiments that determine the isotopic fractionation induced by the conversion of gas-phase nebular oxygen species to solid species, which is a key step in the early evolutionary stage of the solar system.

Here, we present results of the measurement of oxygen isotopic compositions of SiO₂ solids generated via gas-phase reactions under controlled experimental conditions. In these experiments, ultrahigh purity (UHP) SiO nuggets were lased with an excimer laser [Lambda Physik Compex 110 (Coherent Incorporated, Santa Clara, California), KrF, 248 nm] inside a vacuum chamber at two different initial conditions: set I, in the presence of UHP O₂, and set II, in mixtures of UHP O₂ and H₂ of differing proportions. Lasing of SiO generates a plume of neutral SiO gas (see supplementary materials), which reacts with the gases inside the chamber to form silicon dioxide particles throughout the chamber. In set III experiments, the product SiO₂ is formed via both mechanisms of set I and set II (as subsets) experiments, and the products were collected for analysis. Scanning electron microscopic analysis provided the stoichiometry of SiO₂ for the product solids for all cases (fig. S2).

The measured oxygen isotopic compositions of SiO₂ formed in set I-type experiments (without H₂) show MD fractionation, whereas those produced in set II-type experiments (with H₂) reflect MI fractionation (Fig. 1 and table S1). The corresponding isotopic compositions of the residual oxygen reservoirs are fractionated in MD and MI fashion, respectively, for set I and set II experiments (fig. S3 and tables S2 and S3). The extent of the MI component in SiO₂, measured by $\Delta^{17}\text{O}$ ($=\delta^{17}\text{O} - 0.516 \times \delta^{18}\text{O}$), increases with increasing initial H₂/O₂ ratio, and the maximum $\Delta^{17}\text{O}$ value was ~1.7‰, measured at an H₂/O₂ ratio of 25.6. The higher ambient gas pressure suppresses the expansion of the laser plume and also rapidly cools the plume by collisional deactivation limiting the extent of oxidation in the higher ratio and pressure experiments (see supplementary text).

Comparing the expected oxidation reactions 1 to 4 and 1 to 10 for set I and set II experiments

(Table 1), respectively, and their corresponding experimental results (Fig. 1, fig. S3, and tables S1 to S3), we suggest that the observed MI composition in the residual oxygen and product SiO₂ may not be due to ozone formation [known to generate a MI composition (9)]. Set I-type ex-

periments are the most oxidative and kinetically favor ozone formation compared with set II types. The results from set I experiments are strictly MD (Fig. 1 and fig. S3) and cannot involve ozone formation. Analyzing the entire data set, we observed a MI composition only when H₂ is

Fig. 1. Measured oxygen isotopic compositions in three-isotope plot. The compositions of product SiO₂ from set I- and set II-type experiments in set III are shown in blue squares and circles, respectively, with respect to the initial SiO composition. Compositions of the starting SiO and oxygen and product SiO₂ from set I-type experiments all lie on a line with a MD slope of 0.516. The SiO₂ formed in set II-type experiments show MI compositions with a slope value of 0.6. The calculated isotopic compositions of SiO₂ formed by group 2 reactions (OH dominated, see supplementary text) lie on a regression line with a slope value of 1.09 ± 0.1 . The standard deviation of data presented here are $\pm 0.2\text{‰}$ (much smaller than the size of the symbols).

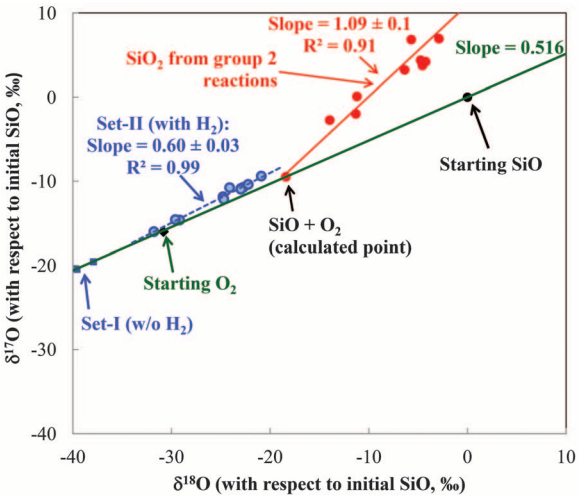
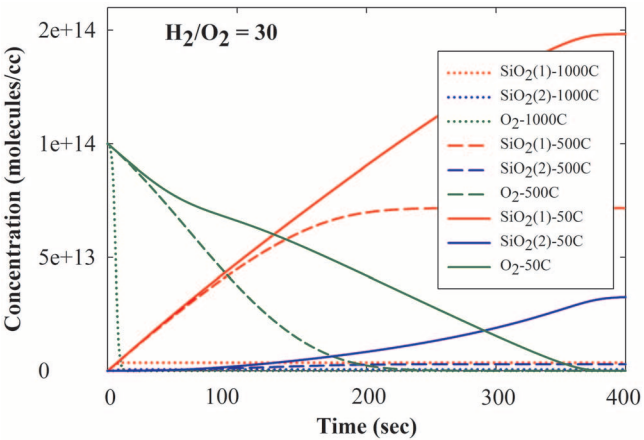


Table 1. List of relevant reactions. Relevant reactions for set I and set II experiments.

Set I-type experiments	Set II-type experiments	Reaction number
	Group 1	
SiO + O ₂ → SiO ₂ + O	SiO + O ₂ → SiO ₂ + O	1
SiO + O → SiO ₂	SiO + O → SiO ₂	2
O ₂ + hv → O + O	O ₂ + hv → O + O	3
O + O ₂ + M → O ₃ + M	O + O ₂ + M → O ₃ + M	4
	Group 2	
	O + H ₂ + M → OH + H + M	5
	OH + H ₂ → H ₂ O + H	6
	SiO + OH → SiO ₂ + H	7
	SiO + H ₂ O → SiO ₂ + OH	8
	H + O ₂ + M → HO ₂ + M	9
	SiO + HO ₂ → SiO ₂ + OH	10

Fig. 2. Results of kinetic model simulation. Time-dependent change in concentrations of SiO₂ formed via group 1 and group 2 reactions and the concentration of O₂ for the simulation run with H₂/O₂ ratio of 30 is shown for three different temperatures. The SiO₂ amount measured in the experiments best matched the simulation run of effective chamber temperature of 50°C. Although the temperature of the laser plume is high (>1000°C), the estimated volume of the laser plume is only ~1/100 of the chamber volume, and, therefore, the match at lower temperature (50°C) with the experiment is reasonable. The result shows that, in set II-type experiments, group 1 reactions are the dominant source of SiO₂ formation, whereas group 2 reactions contribute only 11 to 18% of the total SiO₂.



present, requiring reactions 7, 8, and 10 to be the source of the MI composition.

Because reaction 9 is a MI process (13), there is a possibility that the isotopic composition measured in these experiments was acquired during oxidation by HO_2 , a product of reaction 9, which possesses a MI composition. To test HO_2 reaction channels, we performed photolysis of a mixture of O_2 and H_2 in a glass chamber (without SiO) with ultraviolet photons from the same laser as a control. The residual oxygen was collected (after separating it from H_2 and water), and the oxygen isotopic composition was determined to be MD (fig. S3), indicating that the MI character measured in the set II experiments does not originate from HO_2 species via reaction channel 9.

The oxidation of CO by OH produces CO_2 of MI composition (14, 15); thus reaction 7 ($\text{SiO} + \text{OH} \rightarrow \text{SiO}_2 + \text{H}$) may be the source of the measured MI effect because both CO and SiO possess a homologous valence electron configuration in their ground states (16). On the basis of symmetry considerations, it has been proposed that a gas-phase MI effect may arise from the reaction $\text{O} + \text{SiO} \rightarrow \text{SiO}_2$ (17). An analogous reaction, $\text{O} +$

$\text{CO} \rightarrow \text{CO}_2$, has experimentally demonstrated notable mass-independently fractionated products (18, 19). However, the results from set I experiments do not reflect the fractionation effects of this process. In set I experiments, SiO_2 formed through reactions 1 and 2 may have a potential for isotope exchange between O_2 and O , and the original isotopic signature (MI) from reaction 2 (e.g., $\text{O} + \text{SiO} \rightarrow \text{SiO}_2$) may be removed by exchange, as well as by the MD SiO_2 produced by reaction 1 (e.g., $\text{O}_2 + \text{SiO} \rightarrow \text{SiO}_2 + \text{O}$).

A vibrationally excited transition state (COOH^*) has been predicted for the $\text{CO} + \text{OH} \rightarrow \text{CO}_2 + \text{H}$ reaction (20–22), and the existence of a vibrationally excited intermediate was demonstrated (23). A similar intermediate state (SiOOH^*) for the $\text{OH} + \text{SiO} \rightarrow \text{SiO}_2 + \text{H}$ reaction is feasible, and it is via this excited state that the observed MI effect would be generated. The relevant source would be the deactivation of intermediate states leading to isotope selectivity in the product phase, as is the case for ozone. There is no overall agreement on the mechanism for ozone formation, although symmetry is generally considered to be the ultimate source (24).

SiO_2 samples collected in set II-type experiments are formed via multiple reaction channels (Table 1). We developed a chemical kinetic network model including 17 different species and 30 different reactions relevant to the experiments. Time-dependent concentration variations of different species were recorded in this model by setting up appropriate differential equations (see supplementary text). The simulation solutions depict that a significant proportion of SiO_2 (82 to 89%) is produced via oxidation in group 1 reactions (by O_2 and O ; MD in nature) with the remaining channel produced by group 2 oxidation reactions (Fig. 2 and table S4). The model production of SiO_2 via H_2O_2 oxidation is negligible and consequently not specifically listed in Table 1. Because the contributions from group 2 reactions in the total production of SiO_2 are much smaller (<18%), the measured MI composition in set II-type experiments are diluted by MD contributions from group 1 reactions (Fig. 1).

By considering the fractionations for the group 1 reactions to be those measured in set I reactions and the above-mentioned fractions (of product from groups 1 and 2), we performed an isotopic mass-balance calculation to determine the compositions of SiO_2 formed via group 2 reactions (see supplementary text), which define a line of slope = 1.09 ± 0.1 (1- σ SD) passing through the calculated SiO_2 composition ($\delta^{18}\text{O} = -18.4\text{‰}$, $\delta^{17}\text{O} = -9.5\text{‰}$) formed by the measured $\text{SiO} + \text{O}_2$ reaction (Fig. 1 and supplementary text). The scatter in the calculated data set may be due to the use of a simplified treatment by grouping the reactions in two, where there are six different reactions in group 2, and, based on kinetics, they have different yields at different subsets of experiments because of variation in pressures (table S3). The kinetic simulation shows that among all group 2 reactions (which contribute <18% of total SiO_2 production) more than 90% of SiO_2 forms via OH oxidation. It may be surmised from the observations that the $\text{SiO} + \text{OH}$ reaction follows a slope close to unity.

SiO is a relevant solar nebular species detected in young protoplanetary nebulae and other astronomical objects (25, 26) and is oxidized by OH in the early nebula (27–29). It is plausible that, in the hot (>1000 K) inner solar nebula, SiO oxidation through OH is an initiating reaction in solid silicate formation. Our results demonstrate that the MI composition of meteoritic silicates could be initially generated via OH oxidation of the more reduced SiO , which proceeds along a slope one line. However, transiting through an intermediate excited state is not equilibrium in nature, and the traditional isotopic fractionation calculated on the basis of reduced partition functions (5) diminishes at high temperature limits and is not applicable.

Earth's atmosphere may be isotopically relevant in comparison to the solar nebula because both systems have oxygen reservoirs consisting of relative positive and negative MI anomalies (Fig. 3, A and B). The oxygen-bearing species

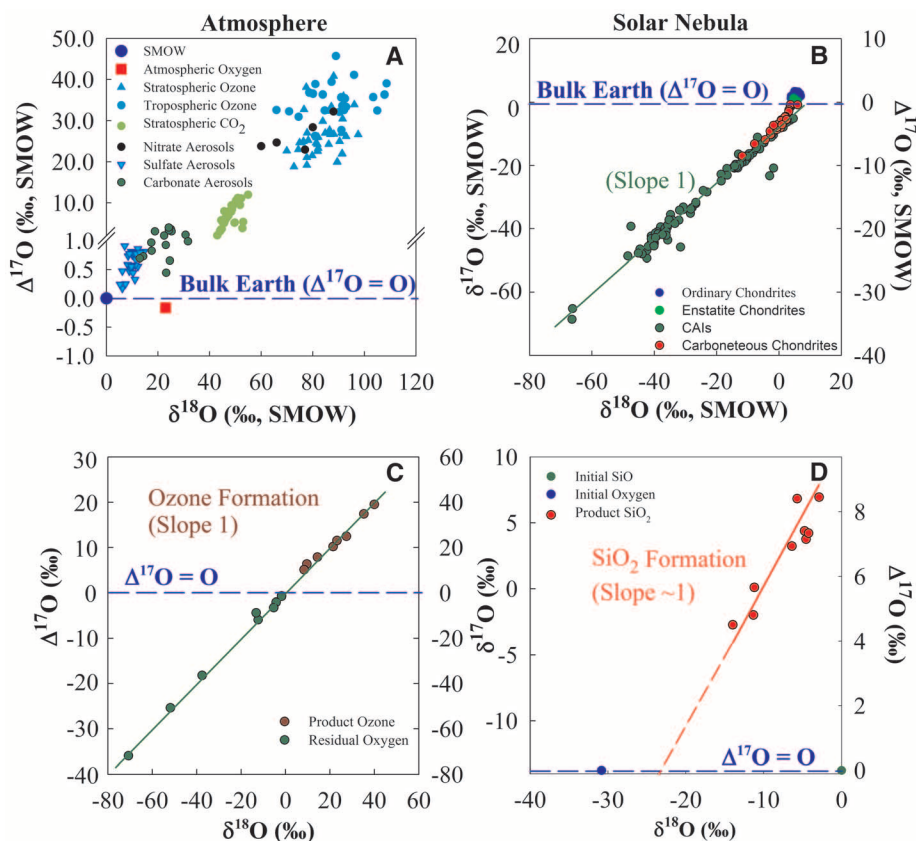


Fig. 3. Similarity of oxygen isotopic distribution in atmosphere and meteorites. Left and right panels represent Earth's atmosphere and meteorites, respectively. (A) The existence of positive and negative $\Delta^{17}\text{O}$ reservoirs in the atmosphere. (B) The presence of positive and negative $\Delta^{17}\text{O}$ reservoirs in the solar nebula (over a slope of ~ 1 line). (C) The composition of product ozone and residual oxygen for the ozone formation experiment in the laboratory. (D) The compositions of SiO_2 formed in the present experiments. The δ values are normalized with starting oxygen and SiO compositions, respectively, for (C) and (D). See supplementary materials for a full list of references used for this compilation.

acquire MI signatures from the ozone formation reaction (9) (Fig. 3C) via atomic oxygen interaction, creating small reservoirs with large positive $\Delta^{17}\text{O}$ and vice versa (e.g., atmospheric O_2 with negative $\Delta^{17}\text{O}$). Similarly, in meteorites, a large negative MI effect in minor phases (CAIs, chondrules and matrices in carbonaceous chondrites, and Ureilites) and a smaller positive MI effect in the more abundant classes (by mass, for example, ordinary chondrites and Rumaruti classes) are observed. Meteoritic negative and positive $\Delta^{17}\text{O}$ reservoirs could have been originated during the actual gas-to-particle formation process as experimentally demonstrated here (Fig. 3D). Solar system oxygen is more complicated, and the initial solar nebular bulk composition is inadequately defined. The measured oxygen composition of solar wind from Genesis concentrator is enriched in ^{16}O compared with CAIs (30), but the oxygen isotopic fractionation between the solar photosphere and the solar wind is not well established and presently an open question.

The final step in the formation of solid silicate from the gas-dominated nebula is a chemical reaction, and the fractionation occurring in this process should be considered regardless of which model is invoked. This reaction, if it occurs in a solar nebula at high temperature, the effect may be larger than observed in the present experiments given the inverse temperature dependency as observed for ozone (31). Last, the observed MI effect is unique to oxygen. The terminal atom regulates the formation and stabilization of vibrationally excited symmetrically structured molecules (31, 32), and oxygen plays this critical role

in SiO_2 . This accounts for why other isotope systems (e.g., Si) do not correlate with oxygen and produce an observable symmetry-dependent fractionation. Carbon and hydrogen are candidates but only possess two stable isotopes and cannot prove the effect, and sulfur, with multiple valence states and exchangeability, is not ideal for preserving the effect.

References and Notes

- R. N. Clayton, L. Grossman, T. K. Mayeda, *Science* **182**, 485–488 (1973).
- J. N. Connelly *et al.*, *Science* **338**, 651–655 (2012).
- R. N. Clayton, *Annu. Rev. Earth Planet. Sci.* **35**, 1–19 (2007).
- M. H. Thieme, *Annu. Rev. Earth Planet. Sci.* **34**, 217–262 (2006).
- H. C. Urey, *J. Chem. Soc.* **1947**, 562–581 (1947).
- M. F. Miller, *Geochim. Cosmochim. Acta* **66**, 1881–1889 (2002).
- J. R. Lyons, E. D. Young, *Nature* **435**, 317–320 (2005).
- J. Bally, W. D. Langer, *Astrophys. J.* **255**, 6 (1982).
- M. H. Thieme, J. E. Heidenreich 3rd, *Science* **219**, 1073–1075 (1983).
- J. E. Heidenreich, M. H. Thieme, *J. Chem. Phys.* **84**, 2129 (1986).
- Y. Q. Gao, R. A. Marcus, *Science* **293**, 259–263 (2001).
- R. A. Marcus, *J. Chem. Phys.* **121**, 8201–8211 (2004).
- J. Savarino, M. H. Thieme, *J. Phys. Chem. A* **103**, 9221–9229 (1999).
- T. Röckmann *et al.*, *Science* **281**, 544–546 (1998).
- A. K. Huff, M. H. Thieme, *Geophys. Res. Lett.* **25**, 3509–3512 (1998).
- E. Bouisset *et al.*, *J. Phys. At. Mol. Opt. Phys.* **24**, 1609–1614 (1991).
- M. H. Thieme, *Science* **283**, 341–345 (1999).
- S. K. Bhattacharya, M. H. Thieme, *Z. Natur. Teil A* **44**, 435 (1989).
- A. Pandey, S. K. Bhattacharya, *J. Chem. Phys.* **124**, 234301 (2006).
- M. Alagia, N. Balucani, P. Casavecchia, D. Stranges, G. G. Volpi, *J. Chem. Phys.* **98**, 8341 (1993).
- J. Li *et al.*, *J. Chem. Phys.* **136**, 041103 (2012).
- X. Song, J. Li, H. Hou, B. Wang, *J. Chem. Phys.* **125**, 6 (2006).
- M. Brouard, D. W. Hughes, K. S. Kalogerakis, J. P. Simons, *J. Phys. Chem. A* **102**, 9559–9564 (1998).
- M. H. Thieme, S. Chakraborty, G. Dominguez, *Annu. Rev. Phys. Chem.* **63**, 155–177 (2012).
- S.-H. Cho, J. Kim, *Astron. J.* **144**, 129 (2012).
- V. Bujarrabal, J. Alcolea, P. Planesas, *Astron. Astrophys.* **257**, 14 (1992).
- G. J. MacPherson, A. Boss, *Proc. Natl. Acad. Sci. U.S.A.* **108**, 19152–19158 (2011).
- F. J. Ciesla, J. N. Cuzzi, *Icarus* **181**, 178–204 (2006).
- C. Walsh, T. J. Millar, H. Nomura, *Astrophys. J.* **722**, 1607 (2010).
- K. D. McKeegan *et al.*, *Science* **332**, 1528–1532 (2011).
- Y. Q. Gao, R. A. Marcus, *J. Chem. Phys.* **116**, 137 (2002).
- M. V. Ivanov, D. Babikov, *Proc. Natl. Acad. Sci. U.S.A.*, published online 19 February 2013 (10.1073/pnas.1215464110).

Acknowledgments: The authors thank the three anonymous reviewers for their helpful critical comments, which helped improve the manuscript. S.C. and M.H.T. acknowledge funding support from NASA Cosmochemistry (NNX09AG93G) and Origins in Solar System (NNX11AB39G) programs. We acknowledge G. Dominguez for helping to develop the kinetic model and helpful discussion with members of the Thieme research group. Data described in this paper are presented in supplementary materials.

Supplementary Materials

www.sciencemag.org/content/342/6157/463/suppl/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S4
Tables S1 to S5
References (33–70)

21 June 2013; accepted 11 September 2013
10.1126/science.1242237

Strong Premelting Effect in the Elastic Properties of hcp-Fe Under Inner-Core Conditions

Benjamí Martorell,* Lidunka Vočadlo, John Brodholt, Ian G. Wood

The observed shear-wave velocity V_S in Earth's core is much lower than expected from mineralogical models derived from both calculations and experiments. A number of explanations have been proposed, but none sufficiently explain the seismological observations. Using ab initio molecular dynamics simulations, we obtained the elastic properties of hexagonal close-packed iron (hcp-Fe) at 360 gigapascals up to its melting temperature T_m . We found that Fe shows a strong nonlinear shear weakening just before melting (when $T/T_m > 0.96$), with a corresponding reduction in V_S . Because temperatures range from $T/T_m = 1$ at the inner-outer core boundary to $T/T_m \approx 0.99$ at the center, this strong nonlinear effect on V_S should occur in the inner core, providing a compelling explanation for the low V_S observed.

Earth's inner core is predominantly made of iron (Fe), but it is commonly assumed to contain 5 to 10% Ni (1) and also light elements such as Si, C, and S, ~2 to 3 weight per-

cent in total (1, 2). Seismic wave velocities through the inner core are known, but at present, seismological and mineralogical models for the inner core do not agree (3–9). A major discrepancy between the observed seismic data and current mineralogical models derived from ab initio calculations is that these mineralogical models predict a shear-wave velocity V_S that is up to 30%

greater than the seismically observed values (4, 9, 10). The addition of small quantities of Ni under these conditions does not reduce V_S by a sufficient amount to explain this (9), and although the effect of light elements on the velocities of Fe is not totally clear at inner-core conditions (11, 12), all studies show that light-element effects are too small [$<5\%$ in V_S for 7% molar fraction in Si at 5000 K and $13,000 \text{ kg m}^{-3}$ (11)] to solve the discrepancy.

Another possible cause of the discrepancy between mineralogical models and seismic data is that the elastic constants of Fe may soften drastically and nonlinearly very near to its melting point T_m , as has been observed in other metals. For instance, the shear modulus of Sn has been experimentally and theoretically shown to decrease by more than 50% at temperatures within ~1% of its melting point (13, 14). According to ab initio simulations, the melting point of pure Fe at the conditions of the inner core is in the range 6200 to 6900 K (15–17) according to phase coexistence calculations (solid and liquid), with upper limit estimates up to 7500 K (18) when only the solid phase is heated until melting. The highest temperature for which the elastic properties of hcp-Fe have been obtained computationally is 6000 K (5); however, relative to the melting

Department of Earth Sciences, University College London, London WC1E 6BT, UK.

*Corresponding author. E-mail: b.massip@ucl.ac.uk

point of this simulation, T/T_m is probably ~ 0.8 and so this temperature is likely to be too low to reveal any strong elastic shear weakening just below melting.

To examine whether premelting effects in the elasticity of Fe can resolve the discrepancy in V_S between mineralogy and seismology, we simulated the effect of temperature on V_S at 360 GPa for hcp-Fe up to its melting point. We performed periodic ab initio calculations based on density functional theory (DFT) derived from quantum mechanics, coupled with molecular dynamics to obtain the elastic properties of hcp-Fe at finite temperatures (19).

Simulations of hcp-Fe at 360 GPa and at temperatures of 6600, 7000, 7250, and 7340 K (19), together with previous simulations at the same pressure and lower temperatures (9), indicated that the behavior of the elastic constants up to ~ 6600 K (Fig. 1) is very similar to that found in our earlier work on hcp-Fe at ~ 315 GPa and temperatures up to 5500 K (20). Specifically, elastic constants [defined as the ratio of applied stress on a material to the strain produced (19)] c_{11} , c_{33} , and c_{44} decreased with temperature, and c_{12} and c_{13} slightly increased (Fig. 1). However, one important difference between the simulations at 360 GPa and 315 GPa is that at higher pressure c_{33} is always larger than c_{11} ; this finding suggests a large pressure dependence for the c_{11} - c_{33} crossover.

Above 6600 K, our calculations show that all of the elastic constants decreased with temperature, with some of them displaying very strong temperature dependence (Fig. 1). In particular, c_{44} , c_{12} , and c_{11} dropped by 46%, 19%, and 32%, respectively, from 7000 to 7340 K. This pronounced drop for a temperature increase of only 340 K indicates that the calculations above 7000 K are approaching the melting point of the simulated system. Analysis of the radial distribution functions and the root-mean-square displacements of the atoms, however, confirmed that the system remained completely solid during the simulation at 7340 K (fig. S1). A simulation at 8000 K melted completely after 16 ps (19).

The temperature dependence of the shear modulus (G) reveals an almost linear decrease up to 7000 K, followed by an abrupt drop beyond this point (fig. S2). Most models for G versus T de-

scribe only the linear region [e.g., the mechanical threshold stress model or the Steinberg-Cochran-Guinan model (21, 22)] and do not describe its behavior close to the melting temperature. For this reason, Nadal and Le Poac [NP (13)] implemented a new model, based on Lindemann melting theory, that accounts for both the linear region and the region close to the melting temperature. Using this model (19), we obtained a Lindemann coefficient of $f = 0.112$ and a melting temperature of 7350 K for hcp-Fe at 360 GPa. The f coefficient is a material-dependent parameter and is normally between 0.1 and 0.3 (23); hence, our value falls in a reasonable range.

We note that the melting temperature obtained in this way is ~ 850 K higher than that expected from previous ab initio simulations (16, 17), which used the phase coexistence method. This reflects the fact that the goal of the present work is not to obtain an accurate estimate for the melting temperature of hcp-Fe at 360 GPa, but rather to investigate the behavior of its elastic constants (and therefore the seismic velocities) very close to melting. To obtain the elastic constants, our simulations needed to be performed on a system with no preexisting surface or defects (such as the solid-liquid interface required for the phase coexistence approach), and it is well known that melting temperatures obtained in such a homogeneous system (mechanical melting) are substantially higher than the true thermodynamic (or heterogeneous) melting temperature (24, 25). The melting temperature obtained here using the Nadal-Le Poac model is about 15% higher than that obtained using free energies or phase coexistence methods and is in accord with previous work showing that homogeneous melting temperatures are about 20% higher than the heterogeneous melting temperatures (26–31).

Although the strong decrease in elastic moduli in Fe observed here is seen close to the homogeneous melting temperature, there is good evidence that this also happens in a real heterogeneous sample. First, the experimentally measured elastic constants of Sn show a strong weakening at $T/T_m \approx 0.99$, where T_m is the true heterogeneous melting temperature. Second, this decrease has also been observed in atomistic simulations in body-centered cubic (bcc) vanadium at $T/T_m \approx 0.98$ (24). Third, the strong elastic weakening is

associated with a rapid increase in defects (defined as over- or undercoordinated atoms), and this occurs at both the homogeneous and heterogeneous melting temperatures (27–31). The only difference in the heterogeneous case is that surface and preexisting defects can propagate into the bulk at a lower temperature than in a homogeneous solid. The number of atomic defects in our simulation jumped from 34% at 7340 K to 70% at 8000 K (19) (fig. S3). This is in very good agreement with previous simulations on much larger systems (27–31). Thus, it is relative temperature (T/T_m) rather than absolute temperature that is important.

Both compressional-wave (V_P) and V_S velocities decreased almost linearly with temperature up to ~ 7000 K at 360 GPa, with a substantial drop beyond this point (Fig. 2). The temperatures at which the velocities from the Preliminary Reference Earth Model (PREM) and our NP-like (like NP model but for velocities instead of G) model agree (7130 K for V_P and 7250 K for V_S) are at T/T_m values of 0.971 and 0.988, respectively, relative to the melting temperature of the simulation. Using an adiabatic geotherm (32), we find that the center of Earth should be ~ 200 K hotter than the inner-core boundary (ICB), whereas the melting line at the center of the inner core is 280 K above the temperature at the ICB (17); this leads to a value at the center of the inner core of $T/T_m = 0.988$. Thus, the core does indeed lie in a range of T/T_m where the velocities might be expected to be strongly decreased near melting.

Our results show that V_P and V_S for the inner core can be fitted with pure Fe for a physically sensible value of T/T_m . However, our simulated density of pure Fe is $\sim 3\%$ too high, and so the presence of light elements is still required to match inner-core values (table S1), but we would expect Fe with a few percent light elements to also show a strong shear softening near the melting temperature. If we assume that the light elements reduce the melting temperature of the Fe alloy, then the softening will occur at lower temperatures than in pure Fe, putting it in a more reasonable

Fig. 1. Calculated elastic constants for hcp-Fe as a function of simulation temperature at 360 GPa. The solid black curves are fits to NP-like models (19). The gray band represents the minimum and maximum melting temperatures (18). The points below 6000 K are from (9). (A) The complete temperature range. (B) Results in the nonlinear regime.

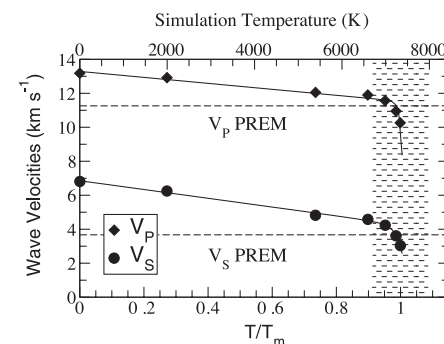
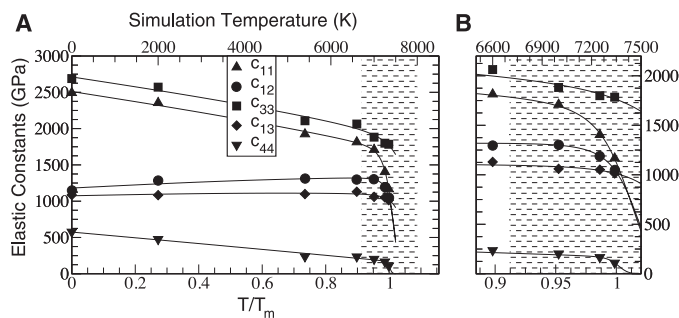


Fig. 2. Calculated V_P and V_S velocities for hcp-Fe as a function of T/T_m and simulation temperature at 360 GPa. The solid black curves are fits to NP-like models (19). The gray band represents the minimum and maximum melting temperatures for hcp-Fe (18). The points below 6000 K are from (9).

range of likely core temperatures. However, further investigations into multicomponent systems are essential to fully understand their effect on the elastic properties of the core. Overall, our results demonstrate that the inner core is likely to be in the strongly nonlinear regime; hence, there is no need to invoke special circumstances such as strong anelasticity, partial melts, or combinations of crystalline phases in order to match the observed seismic velocities and densities of the inner core.

References and Notes

1. F. Birch, *J. Geophys. Res.* **69**, 4377–4388 (1964).
2. J.-P. Poirier, *Phys. Earth Planet. Inter.* **85**, 319–337 (1994).
3. A. Cao, B. Romanowicz, N. Takeuchi, *Science* **308**, 1453–1455 (2005).
4. L. Vočadlo, *Earth Planet. Sci. Lett.* **254**, 227–232 (2007).
5. X. Sha, R. E. Cohen, *Phys. Rev. B* **81**, 094105–094110 (2010).
6. X. Sha, R. E. Cohen, *Geophys. Res. Lett.* **37**, L10302–L10305 (2010).
7. D. Antonangeli et al., *Earth Planet. Sci. Lett.* **225**, 243–251 (2004).
8. A. P. Kantor et al., *Phys. Earth Planet. Inter.* **164**, 83–89 (2007).
9. B. Martorell, J. Brodholt, I. G. Wood, L. Vočadlo, *Earth Planet. Sci. Lett.* **365**, 143–151 (2013).
10. A. M. Dziewonski, D. L. Anderson, *Phys. Earth Planet. Inter.* **25**, 297–356 (1981).
11. D. Antonangeli et al., *Earth Planet. Sci. Lett.* **295**, 292–296 (2010).
12. Z. Mao et al., *Proc. Natl. Acad. Sci. U.S.A.* **109**, 10239–10244 (2012).
13. M.-H. Nadal, P. Le Poac, *J. Appl. Phys.* **93**, 2472–2480 (2003).
14. M.-H. Nadal, C. Hubert, G. Ravel-Chapuis, *J. Alloys Compd.* **444–445**, 265–267 (2007).
15. D. Alfè, G. D. Price, M. J. Gillan, *Phys. Rev. B* **65**, 165118 (2002).
16. D. Alfè, *Phys. Rev. B* **79**, 060101–060104 (2009).
17. E. Sola, D. Alfè, *Phys. Rev. Lett.* **103**, 078501–078504 (2009).
18. G. Morard, J. Bouchet, D. Valencia, S. Mazevet, F. Guyot, *High Energy Density Phys.* **7**, 141–144 (2011).
19. See supplementary materials on Science Online.
20. L. Vočadlo, D. Dobson, I. G. Wood, *Earth Planet. Sci. Lett.* **288**, 534–538 (2009).
21. Y. P. Varshni, *Phys. Rev. B* **2**, 3952–3958 (1970).
22. M. W. Guinan, D. J. Steinberg, *J. Phys. Chem. Solids* **35**, 1501–1512 (1974).
23. D. R. Nelson, *Defects and Geometry in Condensed Matter Physics* (Cambridge Univ. Press, Cambridge, 2002).
24. V. Sorkin, E. Polturak, J. Adler, *Phys. Rev. B* **68**, 174102–174107 (2003).
25. V. Sorkin, E. Polturak, J. Adler, *Phys. Rev. B* **68**, 174103–174109 (2003).
26. K. Lu, Y. Li, *Phys. Rev. Lett.* **80**, 4474–4477 (1998).
27. F. Delogu, *J. Phys. Chem. B* **110**, 12645–12652 (2006).
28. F. Delogu, *J. Phys. Chem. B* **110**, 3281–3287 (2006).
29. F. Delogu, *J. Phys. Condens. Matter* **18**, 5639–5653 (2006).
30. G. Manai, F. Delogu, *Physica B* **392**, 288–297 (2007).
31. G. Manai, F. Delogu, *J. Mater. Sci.* **42**, 6672–6683 (2007).
32. J. P. Poirier, *Introduction to the Physics of the Earth's Interior* (Cambridge Univ. Press, Cambridge, 2000), pp. 230–244.

Acknowledgments: Supported by Natural Environment Research Council grant NE/H003975/1 (L.V.). Calculations were performed in the HECTOR supercomputer facility. Computer code VASP is available at www.vasp.at. The data presented in this paper are given in the main text and in the supplementary materials. B.M. performed research, analyzed data, and wrote the paper. L.V. designed research, analyzed data, and wrote the paper. J.B. analyzed data and wrote the paper. I.G.W. designed the research, analyzed data, and wrote the paper.

Supplementary Materials

www.sciencemag.org/content/342/6157/466/suppl/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S3
Table S1
References (33–43)

23 July 2013; accepted 26 September 2013
Published online 10 October 2013;
10.1126/science.1243651

Atypical Combinations and Scientific Impact

Brian Uzzi,^{1,2} Satyam Mukherjee,^{1,2} Michael Stringer,^{2,3} Ben Jones^{1,4*}

Novelty is an essential feature of creative ideas, yet the building blocks of new ideas are often embodied in existing knowledge. From this perspective, balancing atypical knowledge with conventional knowledge may be critical to the link between innovativeness and impact. Our analysis of 17.9 million papers spanning all scientific fields suggests that science follows a nearly universal pattern: The highest-impact science is primarily grounded in exceptionally conventional combinations of prior work yet simultaneously features an intrusion of unusual combinations. Papers of this type were twice as likely to be highly cited works. Novel combinations of prior work are rare, yet teams are 37.7% more likely than solo authors to insert novel combinations into familiar knowledge domains.

Scientific enterprises are increasingly concerned that research within narrow boundaries is unlikely to be the source of the most fruitful ideas (1). Models of creativity emphasize that innovation is spurred through original combinations that spark new insights (2–10). Current interest in team science and how scientists search for ideas is premised in part on the idea that teams can span scientific specialties, effectively combining knowledge that prompts scientific breakthroughs (11–15).

Yet the production and consumption of boundary-spanning ideas can also raise well-known challenges (16–21). If, as Einstein believed (21), individual scientists inevitably become narrower in their expertise as the body of scientific knowledge expands, then reaching effectively across boundaries may be increasingly challenging (4), especially given the difficulty of searching unfamiliar domains (17, 18). Moreover, novel ideas can be difficult to absorb (19) and communicate, leading scientists to intentionally display conventionality. In his *Principia*, Newton presented his laws of gravitation using accepted geometry rather than his newly developed calculus, despite the latter's importance in developing his insights (22). Similarly, Darwin devoted the first part of the *Origin of Species* to conventional, well-accepted knowledge about the selective breeding of dogs, cattle, and birds. From this viewpoint, the balance

between extending science with atypical combinations of knowledge while maintaining the advantages of conventional domain-level thinking is critical to the link between innovativeness and impact. However, little is known about the composition of this balance or how scientists can achieve it.

In this study, we examined 17.9 million research articles in the Web of Science (WOS) to see how prior work is combined. We present facts that indicate (i) the extent to which scientific papers reference novel versus conventional combinations of prior work, (ii) the relative impact of papers based on the combinations they draw upon, and (iii) how (i) and (ii) are associated with collaboration.

We considered pairwise combinations of references in the bibliography of each paper (23, 24). We counted the frequency of each co-citation pair across all papers published that year in the WOS and compared these observed frequencies to those expected by chance, using randomized citation networks. In the randomized citation networks, all citation links between all papers in the WOS were switched by means of a Monte Carlo algorithm. The switching algorithm preserves the total citation counts to and from each paper and the distribution of these citation counts forward and backward in time to ensure that a paper (or journal) with n citations in the observed network will have n citations in the randomized network. For both the observed and the randomized paper-to-paper citation networks, we aggregated counts of paper pairs into their respective journal pairs to focus on domain-level combinations (24–26). In the data, there were over 122 million potential journal pairs created by the 15,613 journals indexed in the WOS.

¹Kellogg School of Management, Northwestern University, 2001 Sheridan Road, Evanston, IL 60208, USA. ²Northwestern Institute on Complex Systems, Northwestern University, 600 Foster, Evanston, IL 60208, USA. ³Datascopie Analytics, 180 West Adams Street, Chicago, IL 60603, USA. ⁴National Bureau of Economic Research, 1050 Massachusetts Avenue, Cambridge, MA 02138, USA.

*Corresponding author. E-mail: bjones@kellogg.northwestern.edu

Comparing the observed frequency with the frequency distribution created with the randomized citation networks, we generated a z score for each journal pair. This normalized measure describes whether any given pair appeared novel or conventional. Z scores above zero indicate pairs that appeared more often in the observed data than expected by chance, indicating relatively common or “conventional” pairings. Z scores below zero indicate pairs that appear less often in the observed WOS than expected by chance, indicating relatively atypical or “novel” pairings. For example, in the year 1980, the pairing *Tetrahedron* and *Experientia* had a high z score (21.55) indicating a conventional pairing, whereas *Tetrahedron* paired with *Life Sciences* had a negative z score (−17.67), indicating a pairing more unusual than chance. The supplementary materials detail these computations, the null model, and an illustrative example (table S1 and figs. S1 to S3).

As a simple validation of the z score measure, we found that journal pairs from the same WOS disciplinary designation had significantly higher z scores than did interdisciplinary journal pairs (table S3 and fig. S11). At the same time, only a minority (40.1%) of interdisciplinary journal pairs were novel, having z scores below zero

in the 1990s. This pattern indicates that observed journal pairings from the same WOS disciplines tend to be conventional, and interdisciplinary WOS journal pairings are less substantially conventional but still not consistently novel.

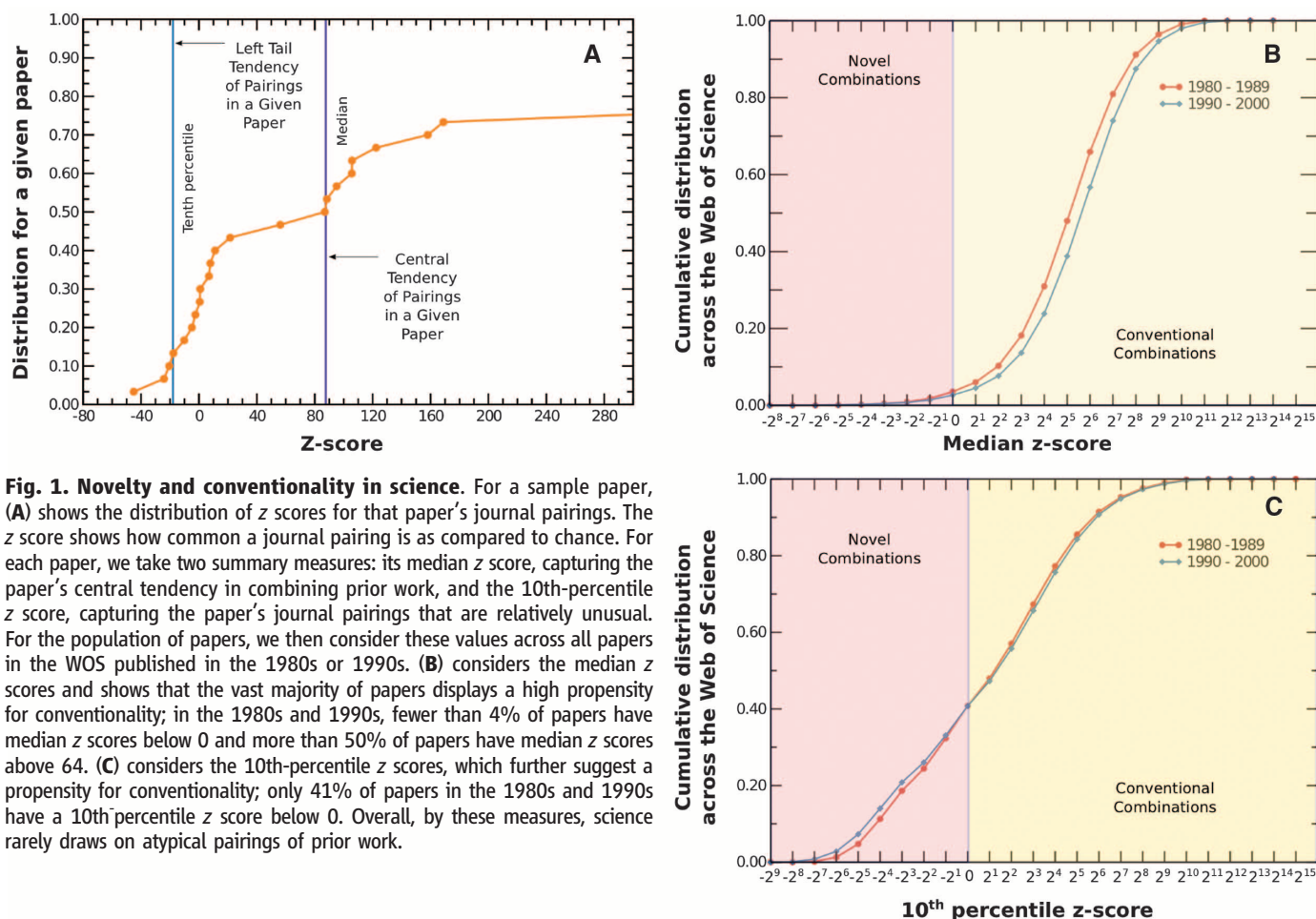
The above method assigns each paper a distribution of journal pair z scores based on the paper’s reference list (Fig. 1A). To characterize a paper’s tendency to draw together conventional and novel combinations of prior work, we examined two summary statistics. First, to characterize the central tendency of a paper’s combinations, we considered the paper’s median z score. The median allows us to characterize conventionality in the paper’s main mass of combinations. Second, we considered the paper’s 10th-percentile z score. The left tail allows us to characterize the paper’s more unusual combinations, where novelty may reside.

We found that papers typically relied on very high degrees of conventionality. Figure 1B presents the distribution of papers’ median z scores for the WOS in the indicated decades. Considering that a z score below zero represents a journal pair that occurs less often than expected by chance, the analysis of median z scores suggests very high degrees of conventionality. Half of the papers have median z scores exceeding

69.0 in the 1980s and 99.5 in the 1990s. Moreover, papers with a median z score below zero are rare. In the 1980s, only 3.54% of papers had this feature, whereas in the 1990s the percentage fell to 2.67%, indicating a persistent and prominent tendency for high conventionality.

Focusing on each paper’s left tail combinations, we found that even among the paper’s relatively unusual journal combinations, the majority of papers did not feature atypical journal pairs. Figure 1C shows that 40.8% of the papers in 1980s and 40.7% in the 1990s have a 10th-percentile z score below zero. Overall, by these measures, science typically relies on highly conventional combinations and rarely incorporates journal pairs that are uncommon compared to chance. Analyses in the supplementary materials (fig. S6) show that these empirical regularities for the WOS taken as a whole are largely replicated on a field-by-field basis and across time.

Our next finding indicates a powerful relationship between combinations of prior work and ensuing impact. Figure 2 presents the probability of a “hit” paper, conditional on the combination of its referenced journal pairs. Hit papers are operationalized as those in the upper 5th percentile of citations received across the whole data set, as measured by total citations through 8 years



after publication (the supplementary materials consider alternative definitions of hit papers). The vertical axis shows the probability of a hit paper conditional on a 2×2 categorization indicating the paper's (i) "median conventionality" (an indicator for whether the paper's median z score is in the upper or lower half of all median z scores)

and (ii) "tail novelty" (an indicator for whether the paper's 10th-percentile z score is above or below zero).

Papers with "high median conventionality" and "high tail novelty" display a hit rate of 9.11 out of 100 papers, or nearly twice the background rate of 5 out of 100 papers. All other categories

show significantly lower hit rates. Papers featuring high median conventionality but low tail novelty displayed hit rates of 5.82 out of 100 papers, whereas those featuring low median conventionality but high tail novelty display hit rates of 5.33 out of 100 papers. Finally, papers low on both dimensions have hit rates of just 2.05 out of 100.

Further analyses suggest a universality of these relationships for scientific work across time and fields. We considered the same relationships for different time periods (fig. S4), for different definitions of high-impact papers (fig. S5), and for each of 243 fields of science (fig. S6 and table S2). These analyses confirmed the findings above. Thus, novelty and conventionality are not opposing factors in the production of science; rather, papers with an injection of novelty into an otherwise exceptionally familiar mass of prior work are unusually likely to have high impact.

Collaboration is often claimed to produce more novel combinations of ideas (10–14), but the extent to which teams incorporate novel combinations across the universe of fields is unknown. Team-authored papers were more likely to show atypical combinations than were single- or pair-authored papers. Figure 3A shows that the distribution of 10th-percentile z scores shifted significantly leftward as the number of authors increased [Kolmogorov-Smirnov (K-S) tests indicate solo versus pair $P = 0.016$, pair versus team $P = 0.001$, team versus solo $P < 0.001$]. Papers written by one, two, or three or more authors showed high tail novelty in 36.1, 39.8, and 49.7% of cases, respectively, indicating that papers with three or more authors showed an increased frequency of high tail novelty over the solo-author rate by 37.7%.

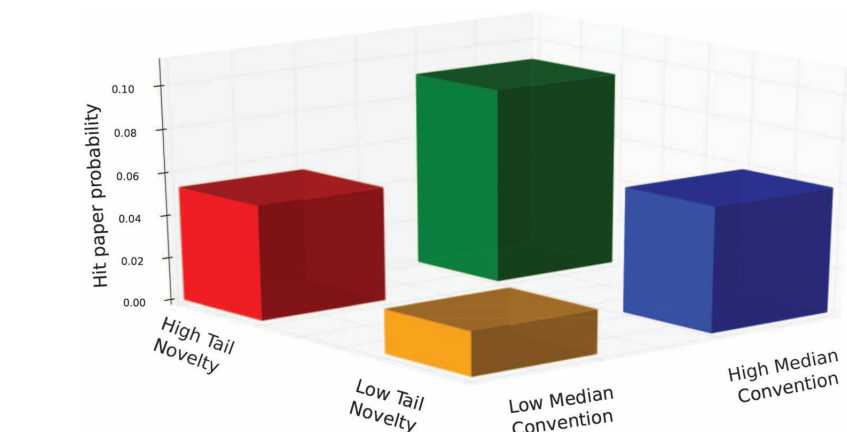


Fig. 2. The probability of a "hit" paper, conditional on novelty and conventionality. This figure presents the probability of a paper being in the top 5% of the citation distribution conditional on two dimensions: whether a paper exhibits (i) high or low median conventionality and (ii) high or low tail novelty, as defined in the text. Papers that combine high median conventionality and high tail novelty are hits in 9.11 out of 100 papers, a rate nearly double the background rate of 5%. Papers that are high on one dimension only (high median conventionality or high tail novelty but not both) have hit rates about half as large. Papers with low median conventionality and low tail novelty have hit rates of only 2.05 out of 100 papers. The sample includes all papers published in the WOS from 1990 to 2000. The supplementary materials show similar findings when considering (i) all other decades from 1950 to 2000; (ii) hit papers defined as the top 1 or 10% by citations; and (iii) analyses controlling for field and other observable differences across papers, hinting at a universality of these relationships for scientific work. The difference in the hit probabilities for each category is statistically significant ($P < 0.00001$). The percentage of WOS papers in each category are: 6.7% (green bar), 23% (gold bar), 26% (red bar), and 44% (blue bar).

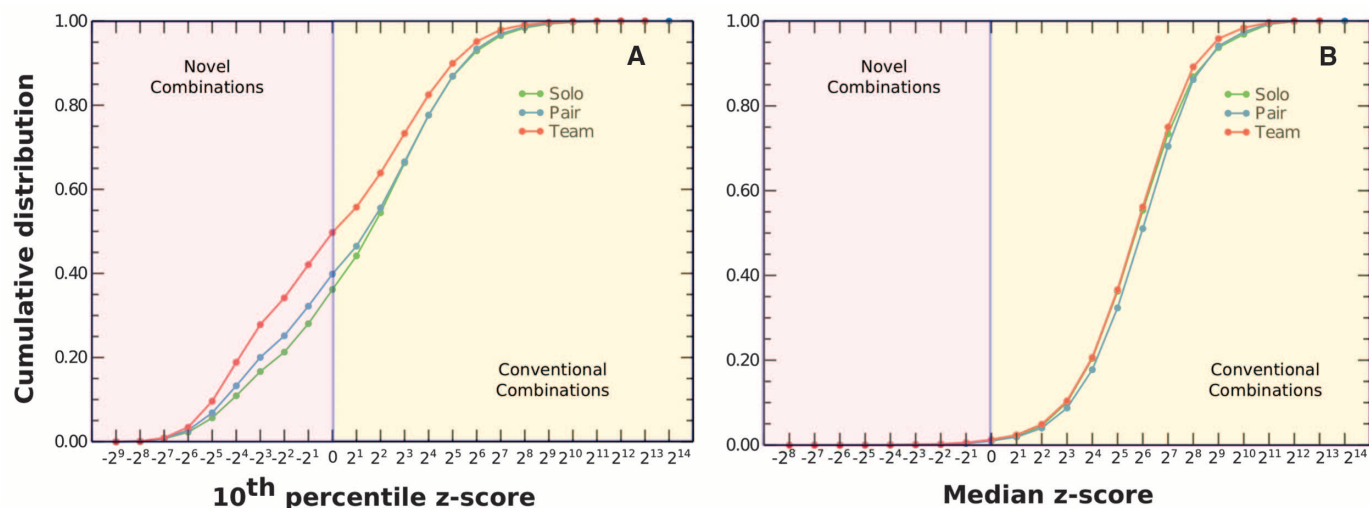


Fig. 3. Authorship structure, novelty, and conventionality. Team-authored papers are more likely to incorporate tail novelty but without sacrificing a central tendency for high conventionality. Papers introduce tail novelty (a 10th-percentile z score less than 0) in 36.2, 39.9, and 49.7% of cases for solo authors, dual authors, and three or more authors, respectively (A). K-S tests confirm that the distributions of tail novelty are

distinct (solo versus pair $P = 0.016$, pair versus team $P = 0.001$, team versus solo $P < 0.001$). In contrast, each team size shows similar distributions for median conventionality [(B), K-S tests indicate no statistically significant differences]. These findings suggest that a distinguishing feature of teamwork, and teams' exceptional impact, reflects a tendency to incorporate novelty.

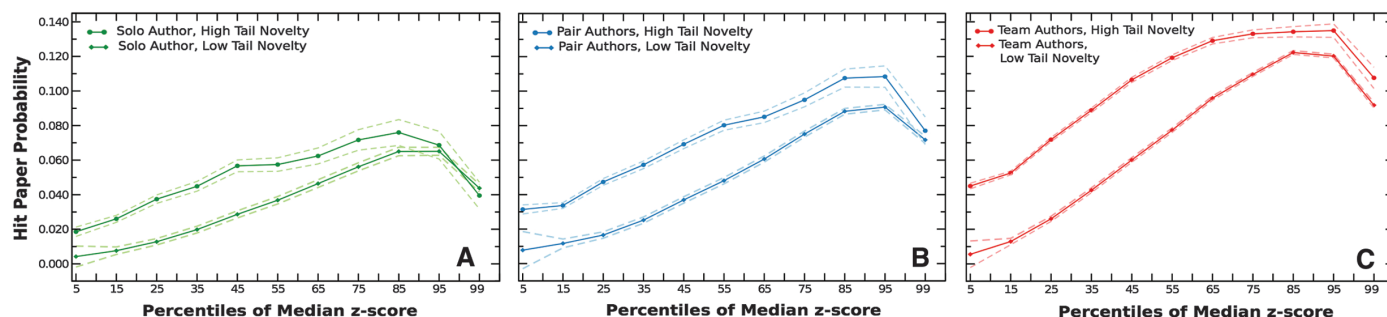


Fig. 4. Novel and conventional combinations in the production of science. (A to C) The interplay between tail novelty, median conventionality, and hit paper probabilities shows remarkable empirical regularities. First, high tail novelty papers have higher impact than low tail novelty papers at (i) any level of conventionality and (ii) regardless of authorship structure. Second, increasing median conventionality is associated with higher impact up to the

85th to 95th percentile of median conventionality, after which the relationship reverses. Third, larger teams obtain higher impact given the right mix of tail novelty and median conventionality. Nonetheless, at low levels of median conventionality and tail novelty, even teams have low impact, further emphasizing the fundamental relationship between novelty, conventionality, and impact in science.

Teams were neither more nor less likely than single authors or pairs of authors to display high median conventionality. Figure 3B indicates no significant statistical difference in the median z-score distributions (K-S tests indicate solo versus pair $P = 0.768$, pair versus team $P = 0.417$, team versus solo $P = 0.164$). Teams thus achieve high tail novelty more often than solo authors. Yet, teams were not simply more novel but rather displayed a propensity to incorporate high tail novelty without giving up a central tendency for high conventionality.

In our final analysis, we examined the interplay between citation, combination, and collaboration using regression methods (Fig. 4). Papers were binned into 11 equally sized categories of median conventionality. A separate regression was run for each category of median conventionality and each team size, with field fixed effects. The supplementary materials detail the regression methodology and present additional confirmatory tests (figs. S7 to S10).

There were three primary findings. First, high tail novelty papers had higher impact than low tail novelty papers, an impact advantage that occurred at any level of conventionality and regardless of authorship structure. Second, peak impact occurred in the 85th to 95th percentile of median conventionality, an exceptionally high level. This peak and its position appeared irrespective of tail novelty/no tail novelty or authorship structure. These generic features suggest fundamental underlying rules relating combinations of prior work to the highest-impact science.

Finally, Fig. 4 indicates that at virtually all mixes of tail novelty and median conventionality, larger teams were associated with higher impact. Thus, whereas teams incorporated the highest impact mixes more frequently (Fig. 3), teams also tended to obtain higher impact for any particular mix (Fig. 4). Nonetheless, despite teams' advantage in citations across virtually all fields of science (12), even teams had low impact at low levels of median conventionality and tail novelty.

Our analysis of 17.9 million papers across all scientific fields suggests that the highest-impact

science draws on primarily highly conventional combinations of prior work, with an intrusion of combinations unlikely to have been joined together before. These patterns suggest that novelty and conventionality are not factors in opposition; rather, papers that mix high tail novelty with high median conventionality have nearly twice the propensity to be unusually highly cited.

These findings have implications for theories about creativity and scientific progress. Combinations of existing material are centerpieces in theories of creativity, whether in the arts, the sciences, or commercial innovation (2–4, 6–10, 16). Across the sciences, the propensity for high-impact work is sharply elevated when combinations of prior work are anchored in substantial conventionality, not novelty, while mixing in a left tail of combinations that are rarely seen together. In part, this pattern may reflect advantages to being within the mainstream of a research trajectory, where scientists are currently focused, while being distinctive in one's creativity. Combinations of prior work also relate to “burden of knowledge” theory, which emphasizes the growing knowledge demands on scientists (4, 17, 21). New articles indexed by the WOS now exceed 1.4 million per year across 251 fields, encouraging specialization and challenging scientists' capacity to comprehend new thinking across domains. The finding that teams preserve high conventionality yet introduce tail novelty suggests that teams help meet the challenge of the burden of knowledge by balancing domain-level depth with a capacity for atypical combinations.

This methodology considered paper and journal pairings but can be applied at the level of disciplines, papers, or topics within papers, allowing the examination of combinations of prior work at different resolutions in future studies of creativity and scientific impact. Beyond science, links between novelty and conventionality in successful innovation also appear. E-books retain page-flipping graphics to remind the reader of physical books, and blue jeans were designed with a familiar watch pocket to look like conventional trousers. From this viewpoint, the balance

between extending technology with atypical combinations of prior ideas while embedding them in conventional knowledge frames may be critical to human progress in many domains. Future research questions also arise from our findings. Science is dynamic, with research areas shifting and new fields arising. Although we find that the regularities relating novelty, conventionality, and impact persist across time and fields, understanding how research trajectories shift and how new fields are born are questions that measures of novelty and conventionality may valuably inform. At root, our work suggests that creativity in science appears to be a nearly universal phenomenon of two extremes. At one extreme is conventionality and at the other is novelty. Curiously, notable advances in science appear most closely linked not with efforts along one boundary or the other but with efforts that reach toward both frontiers.

References and Notes

- Committee on Facilitating Interdisciplinary Research, *Facilitating Interdisciplinary Research* (National Academies Press, Washington, DC, 2004).
- H. S. Becker, *Art Worlds* (Univ. of California Press, Berkeley, CA, 1982).
- R. Guimerà, B. Uzzi, J. Spiro, L. A. Amaral, *Science* **308**, 697–702 (2005).
- B. Jones, *Rev. Econ. Stud.* **76**, 283–317 (2009).
- B. F. Jones, S. Wuchty, B. Uzzi, *Science* **322**, 1259–1262 (2008).
- J. Schumpeter, *Business Cycles* (McGraw-Hill, New York, 1939).
- A. P. Usher, *A History of Mechanical Invention* (Harvard Univ. Press, Cambridge, MA, 1954).
- M. L. Weitzman, *Q. J. Econ.* **113**, 331–360 (1998).
- M. Schilling, *Creat. Res. J.* **17**, 131–154 (2005).
- B. Uzzi, J. Spiro, *Am. J. Sociol.* **111**, 447–504 (2005).
- H. J. Falk-Krzesinski et al., *Res. Eval.* **20**, 145–158 (2011).
- S. Wuchty, B. F. Jones, B. Uzzi, *Science* **316**, 1036–1039 (2007).
- D. Stokols, K. L. Hall, B. K. Taylor, R. P. Moser, *Am. J. Prev. Med.* **35** (suppl.), S77–S89 (2008).
- S. M. Fiore, *Small Group Res.* **39**, 251–277 (2008).
- J. A. Evans, J. G. Foster, *Science* **331**, 721–725 (2011).
- R. Collins, *The Sociology of Philosophies: A Global Theory of Intellectual Change* (Harvard Univ. Press, Cambridge MA, 1998).
- L. Fleming, *Manage. Sci.* **47**, 117–132 (2001).
- M. Schilling, E. Green, *Res. Policy* **40**, 1321–1331 (2011).
- R. M. Henderson, K. B. Clark, *Admin. Sci. Q.* **35**, 9–30 (1990).

20. P. Azoulay, J. G. Zivin, G. Manso, *Rand J. Econ.* **42**, 527–554 (2011).
21. A. Einstein, *The World as I See It* (Citadel Press, Secaucus NJ, 1949).
22. D. T. Whiteside, *J. Hist. Astron.* **1**, 116–138 (1970).
23. H. Small, *J. Am. Soc. Inf. Sci.* **24**, 265–269 (1973).
24. M. J. Stringer, M. Sales-Pardo, L. A. Nunes Amaral, *J. Am. Soc. Inf. Sci. Technol.* **61**, 1377–1385 (2010).
25. M. Stringer, M. Sales-Pardo, L. A. Nunes Amaral, *PLoS One* **3**, e1683 (2008).
26. S. Itzkovitz, R. Milo, N. Kashtan, G. Ziv, U. Alon, *Phys. Rev. E Stat. Nonlin. Soft Matter Phys.* **68**, 026127 (2003).

Acknowledgments: Sponsored by the Northwestern University Institute on Complex Systems and by the Army Research Laboratory under Cooperative Agreement Number W911NF-09-2-0053 and Defense Advanced Research Projects Agency grant BAA-11-64, Social Media in Strategic Communication. The views and conclusions contained in this document are those of the authors and should not be interpreted as representing the official policies, either expressed or implied, of the Army Research Laboratory or the U.S. government. All our summary statistics and programs are freely available on request. Our access to the WOS comes through a contract with Thomson Reuters that forbids redistribution of their database; researchers who desire the

raw data on which to run our analytics can obtain it via a paid subscription to Thomson Reuters.

Supplementary Materials

www.sciencemag.org/content/342/6157/468/suppl/DC1
Data and Methods
Supplementary Text
Figs. S1 to S11
Tables S1 to S3
References (27, 28)

14 May 2013; accepted 20 September 2013
10.1126/science.1240474

A Radical Intermediate in Tyrosine Scission to the CO and CN[−] Ligands of FeFe Hydrogenase

Jon M. Kuchenreuther,^{1*} William K. Myers,^{1*} Troy A. Stich,¹ Simon J. George,¹
Yaser Nejatyjahromy,¹ James R. Swartz,² R. David Britt^{1†}

The radical *S*-adenosylmethionine (SAM) enzyme HydG lyses free L-tyrosine to produce CO and CN[−] for the assembly of the catalytic H cluster of FeFe hydrogenase. We used electron paramagnetic resonance spectroscopy to detect and characterize HydG reaction intermediates generated with a set of ²H, ¹³C, and ¹⁵N nuclear spin-labeled tyrosine substrates. We propose a detailed reaction mechanism in which the radical SAM reaction, initiated at an N-terminal 4Fe-4S cluster, generates a tyrosine radical bound to a C-terminal 4Fe-4S cluster. Heterolytic cleavage of this tyrosine radical at the C_α-C_β bond forms a transient 4-oxidobenzyl (4OB[•]) radical and a dehydroglycine bound to the C-terminal 4Fe-4S cluster. Electron and proton transfer to this 4OB[•] radical forms *p*-cresol, with the conversion of this dehydroglycine ligand to Fe-bound CO and CN[−], a key intermediate in the assembly of the 2Fe subunit of the H cluster.

Microbial hydrogenase enzymes catalyze the redox interconversion of protons and H₂ by using earth-abundant metals in their catalytic centers, with NiFe, Fe, and FeFe classes known (1). The FeFe hydrogenases are adept H₂ producers, with turnover frequencies up to 10,000 s^{−1} (2). Their catalytic H cluster (Fig. 1) consists of a conventional 4Fe-4S cluster linked to a unique 2Fe cluster that has two CN[−] ligands, three CO ligands, and a dithiolate bridge with a central atom X, the chemical identity of which is ambiguous in current x-ray structures (3, 4). However, a nitrogen assignment to atom X is supported by recent reports of the assembly of active FeFe hydrogenase by incorporation of a synthetic 2Fe subcluster with an azadithiolate bridge into apoenzyme (5, 6).

The HydE, HydF, and HydG maturases are Fe-S cluster-containing accessory proteins involved in the biological synthesis of the 2Fe component of the H cluster (7). HydE and HydG are members of the ever-growing family of radical *S*-adenosylmethionine (SAM) enzymes, characterized by a CysX₃CysX₂Cys motif, where the three

cysteine residues coordinate a 4Fe-4S cluster through ligation of three Fe ions. The fourth Fe of the cluster binds SAM as a N/O chelate, and SAM is reductively cleaved to produce methionine plus a strongly oxidizing 5-deoxyadenosyl (5'-dA[•]) radical (8, 9). A diverse array of reactions is powered by the H-atom abstraction capability of the resultant 5'-dA[•], and current bioinformatics surveys reveal almost 50,000 members of the radical SAM enzyme class (10).

The focus of this report is the HydG maturase. This radical SAM enzyme generates CO, CN[−], and *p*-cresol by using free tyrosine as its substrate (11–14). Although HydG has yet to be crystallographically characterized, biophysical data indicate that it has two 4Fe-4S clusters (15, 16). The SAM-4Fe-4S cluster is bound near the N terminus, whereas the second cluster is modeled as coordinated to three cysteine residues of a CysX₂CysX₂₂Cys sequence near the C terminus. Sequence homology with other radical SAM enzymes, such as biotin synthase and the tyrosine lyase ThiH, point to HydG having a triose phosphate isomerase barrel structure, in which eight α helices surround a ring composed of eight parallel β strands that contain a buried active site suitable for small-molecule substrates (11, 17).

Like HydG, ThiH also lyses tyrosine, in this case to generate dehydroglycine (DHG) as an intermediate in anaerobic thiamine biosynthesis

(18, 19). ThiH lacks the second 4Fe-4S binding domain of HydG. The electron paramagnetic resonance (EPR) spectrum of the ThiH SAM-[4Fe-4S]⁺ cluster is altered by SAM binding but unaffected by tyrosine incubation. In a proposed mechanism (18, 19), the initial 5'-dA[•] abstracts the phenolic H of the tyrosine substrate, forming a neutral tyrosine radical, which is then cleaved at the C_α-C_β bond. Quantum chemistry calculations favored homolytic cleavage of this bond to form a transient glycy radical, given its lower energy pathway compared with heterolytic cleavage (19). Modeling the differences in reactivity between wild-type HydG and a HydG mutant missing the C-terminal Fe-S cluster (HydG^{SxxS}), which produces some CN[−] but no CO, led Nicolet and co-workers (20) to propose the same glycy radical intermediate in the HydG mechanism, building on the thermodynamic argument for the mechanism in ThiH. However, no radical intermediates have been experimentally characterized for either enzyme.

Here, we report on the EPR spectroscopy of wild-type *Shewanella oneidensis* HydG (HydG^{WT}) expressed in *Escherichia coli*. Such expressed HydG, combined with HydE and HydF, can be used for in vitro synthesis of the 2Fe component of the H cluster and concurrent activation of FeFe hydrogenase apoprotein (14, 21). The use

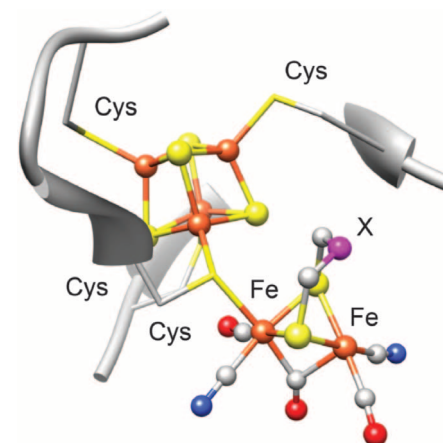


Fig. 1. The catalytic H cluster of FeFe hydrogenases. Ball-and-stick representation (from Protein Data Bank entry 3CY8) was generated by using UCSF Chimera (www.cgl.ucsf.edu/chimera/): Fe (brown), S (yellow), C (gray), O (red), N (blue), and atom X (magenta). H is not shown for simplicity.

¹Department of Chemistry, University of California, Davis, Davis, CA 95616, USA. ²Department of Chemical Engineering and Department of Bioengineering, Stanford University, Stanford, CA 94305, USA.

*These authors contributed equally to this work.

†Corresponding author. E-mail: rdbritt@ucdavis.edu

of tyrosine isotopologs allows us to determine the identity and electronic structure of paramagnetic reaction intermediates, including a previously unrecognized radical species that derives from free tyrosine. On the basis of our results, we propose a mechanistic model for the formation of Fe-coordinated CO and CN^- ligands that are associated with the C-terminal Fe-S cluster of the HydG maturase, a key step in the assembly of the 2Fe subunit of the H cluster.

In order to explore the nature of free tyrosine binding in HydG, we compared both the low-field ($g \gg 2$) (Fig. 2A) and the $g \approx 2$ (Fig. 2B) regions of the X-band EPR spectra of sodium dithionite (DTH)-reduced HydG^{WT} mixed with either L-tyrosine (Tyr) or unreactive *meta*-L-tyrosine (*m*-Tyr), along with the spectrum of the HydG^{SxxxS} mutant devoid of the C-terminal Fe-S cluster (16, 20). In the spectrum of reduced HydG^{WT} without tyrosine or SAM added, the $g \approx 2$ signal (Fig. 2B, i) can be well simulated with a single g

matrix [2.045, 1.936, 1.908] (fig. S1a), and we assign it to the N-terminal [4Fe-4S]⁺ cluster without SAM bound (13, 15). The low-field region of the spectrum (Fig. 2A, i) shows a broad signal, with maximum intensities at $g \approx 9.5$ and $g \approx 5$ that we assign to one or more high-spin (HS) Fe-S cluster forms. The $g \approx 2$ regions for the HydG^{SxxxS} and *m*-Tyr substitutions show an identical signal, which can be simulated with a single g matrix [2.007, 1.878, 1.839] (fig. S1b) and which we assign to the SAM-bound form of the N-terminal [4Fe-4S]⁺ cluster (13). The HS EPR signal is unaltered in the *m*-Tyr sample but completely absent in the mutant HydG^{SxxxS}, indicating that it originates from the C-terminal cluster.

Addition of Tyr to reduced HydG^{WT} (Fig. 2, A and B, iv) results in a large decrease of the HS EPR signal [the remaining $g = 4.3$ signal resembles that of adventitious Fe(III)], along with the addition of new $g \approx 2$ region features, seen mostly as shoulders flanking the N-terminal cluster

signal. The $g \approx 2$ region of the spectrum is simulated with two distinct components: the unaltered N-terminal cluster signal of Fig. 2B, i, plus a new signal with a g matrix [2.060, 1.910, 1.880] (fig. S1c). The loss of the HS EPR signal intensity and the addition of a new $g \approx 2$ component indicates a spin-state conversion triggered by tyrosine binding. 4Fe-4S clusters bound by three cysteines may lose the unique Fe and convert to 3Fe-4S forms, as initially discovered in the aconitase enzyme (22). One such [3Fe-4S]⁺ form, in “purple aconitase” (23) and a related model complex (24), shows g values (9.6 to 4.3) similar to what we observe as the HS EPR signal of HydG before adding tyrosine. The spectral similarities suggest the possibility that the addition of tyrosine substrate to HydG triggers the installation of the unique Fe in the C-terminal cluster, thereby producing the observed $S = 1/2$ [4Fe-4S]⁺ signal. Figure 3 shows a possible model for tyrosine binding to the C-terminal cluster. In step I, tyrosine binds as a N/O chelate to this fourth Fe, analogously to the methionine component of SAM when bound to the N-terminal cluster. This resting-state enzyme model suggests a mechanism in which the C-terminal Fe-S cluster is directly involved in the enzyme activity, along the lines of certain other radical SAM enzymes, such as biotin synthase, where dethiobiotin is bound to a second Fe-S cluster as an intermediate in S-atom transfer (25).

In order to test for such a mechanism, we conducted freeze-quench EPR experiments to trap and characterize paramagnetic intermediates during the HydG reaction. With the addition of DTH, SAM, and tyrosine to initiate the HydG^{WT} reaction (Fig. 2, A and B, v, 30-s time point), the HS C-terminal cluster signal remains small, and the $g \approx 2$ region of the spectrum shows a complex set of peaks in the Fe-S spectral region, plus a new radical signal at $g = 2$. The radical is transient, as measured in samples cryotrapped at varied time points in a set of rapid freeze-quench (RFQ) experiments (Fig. 2C), and a maximum radical EPR signal is observed about 2 s after reaction initiation. Figure 2D shows spectra for longer reaction times. The $g = 2$ radical signal is greatly diminished at time points past 1 min, leaving a complex EPR line shape arising from the two reduced [4Fe-4S]⁺ clusters. Hyperfine sublevel correlation (HYSCORE) and electron nuclear double resonance (ENDOR) spectra (fig. S2) recorded at the prominent $g = 1.9$ feature that forms during the HydG^{WT} reaction show coupled ¹⁵N and ¹³C features (with ¹³C₉, ¹⁵N-Tyr), suggesting that either tyrosine or a tyrosine-derived fragment is bound to the C-terminal 4Fe-4S cluster during the reaction.

We determined the identity of the transient radical signal by using a number of tyrosine isotopologs as substrates in the HydG-catalyzed reaction. Figure 4A shows a set of Q-band (34 GHz) EPR spectra, with the higher frequency chosen to give better resolution of the structured radical EPR signal. The EPR spectrum of the radical trapped in the natural abundance Tyr sample (Fig. 4A, i) is unaltered by ¹³C-labeling at either the C α

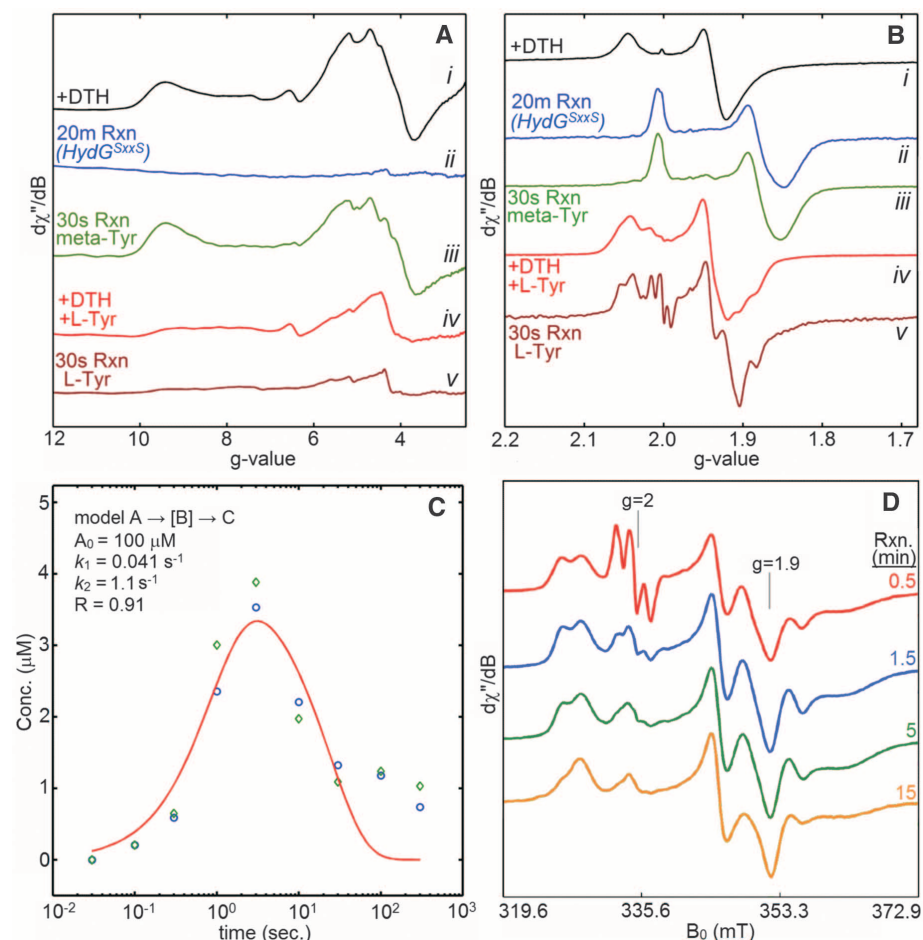


Fig. 2. Continuous-wave (CW) EPR spectra of DTH-reduced HydG samples. (A) Low-field (9.37 GHz, 12 K except as noted, 5 mW) and (B) $g \approx 2$ region (9.37 GHz, 20 K, 0.1 mW) X-band CW EPR spectra of (i) reduced HydG^{WT} without Tyr or SAM, (ii) reduced mutant HydG^{SxxxS} with Tyr and SAM (10 K), (iii) reduced HydG^{WT} with *m*-Tyr and SAM, (iv) reduced HydG^{WT} with Tyr and without SAM, and (v) reduced HydG^{WT} with Tyr and SAM. Rxn, reaction. (C) Kinetics plot of the integrated Q-band radical EPR signal from HydG^{WT} reaction samples trapped by RFQ methods. The red line is a fit to a sequential [A]→[B]→[C] model. (D) X-band (9.37 GHz, 20 K, 0.1 mW) EPR spectra of hand-freeze-quenched reduced HydG^{WT} mixed with Tyr and SAM at varied time points (min).

(Fig. 4A, ii) or the COO^- carbon (not shown), strongly indicating that our cryotrapped radical is not the previously proposed glycyl radical intermediate (20). In contrast, the line shape of the radical collapses with $^2\text{H}_7$, ^{15}N -Tyr (Fig. 4A, iii), a signature that has been used to identify tyrosine protein radicals (26). Neutral tyrosine ($\text{Tyr}^\bullet$) radicals, like those proposed to be produced by the initial phenolic H-atom abstraction in HydG (20), have been widely studied with advanced EPR and electronic structure calculations (27, 28). Figure 4B illustrates the density functional theory (DFT)-derived spin density for such a $\text{Tyr}^\bullet$. There is a large amount of spin density on the phenolic O, as experimentally observed by ^{17}O labeling (28, 29); on C3 and C5, as observed by large hyperfine couplings to their respective protons; and on C1, which transfers large and generally inequivalent hyperfine couplings to the two C_β protons.

The remaining spectra of Fig. 4A show that we have trapped a different radical during the HydG reaction. The spectrum of the $3,5\text{-}^2\text{H}_2$ -Tyr sample (Fig. 4A, iv) is almost identical to that of the natural abundance Tyr sample (Fig. 4A, i), whereas substituting two additional deuterons on C2 and C6 (Fig. 4A, v) causes a dramatic change, showing that electron spin density has shifted from C3 and C5 to C2 and C6. The remaining resolved hyperfine couplings are to the two C_β protons, which show the 1:2:1 pattern diagnostic of two equivalently coupled protons. When these are instead deuterated (Fig. 4A, vi), this 1:2:1 pattern is abolished, and we see the largest effect of all the labeled tyrosines, indicating that the greatest spin density is on C_β . Moreover, there is no observed ^{17}O hyperfine coupling in the $4\text{-}^{17}\text{O}$ -Tyr sample (fig. S3), also inconsistent with a $\text{Tyr}^\bullet$ assignment. Instead, the pattern of hyperfine couplings, as analyzed through DFT-based simulations (fig. S3 and table S1), points to a 4-oxido-benzyl ($4\text{OB}^\bullet$) radical (Fig. 4C) as the origin of the cryotrapped radical EPR signal. This $4\text{OB}^\bullet$, produced by tyrosine $\text{C}_\alpha\text{-C}_\beta$ fragmentation, has its highest spin density at the CH_2 terminus, which is sp^2 -hybridized, giving large and equivalent couplings to these two C_β protons. The overall spin density pattern is shifted by one atom relative to a parent $\text{Tyr}^\bullet$, with higher spin density on C2, C4, and C6 than on C1, C3, and C5 and with a lowered spin density on O (Fig. 4, C versus B). The oxygen-protonated form of the $4\text{OB}^\bullet$ (4-hydroxybenzyl radical) shows a similar calculated shift in its spin density pattern relative to $\text{Tyr}^\bullet$ (fig. S3 and table S1).

Tyrosine binding to the C-terminal cluster and the radical chemistry enabled by the $4\text{OB}^\bullet$ are at the heart of our proposed mechanism (Fig. 3). Although the $\text{HydG}^{\text{Sxxx}}$ mutant does produce some CN^- , stopped flow FTIR spectroscopy shows that this occurs without concomitant generation of CO and on a hundredfold slower time scale than the HydG^{WT} production of Fe-bound CO and CN^- (fig. S4). Thus, the $\text{HydG}^{\text{Sxxx}}$ -derived CN^- is not enzymatically relevant and instead occurs along

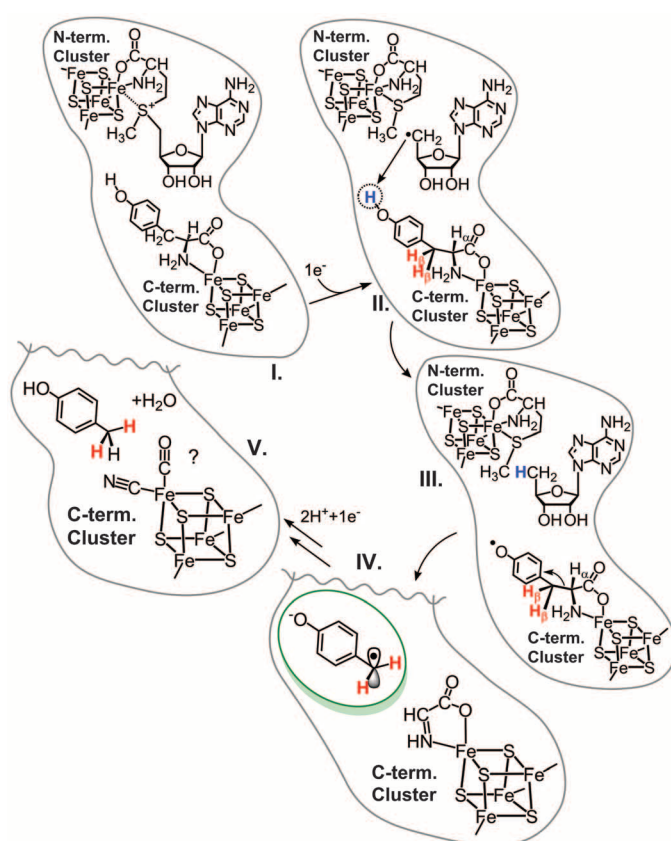


Fig. 3. A proposed mechanism for the HydG-catalyzed production of Fe-bound CO and CN^- ligands from free tyrosine.

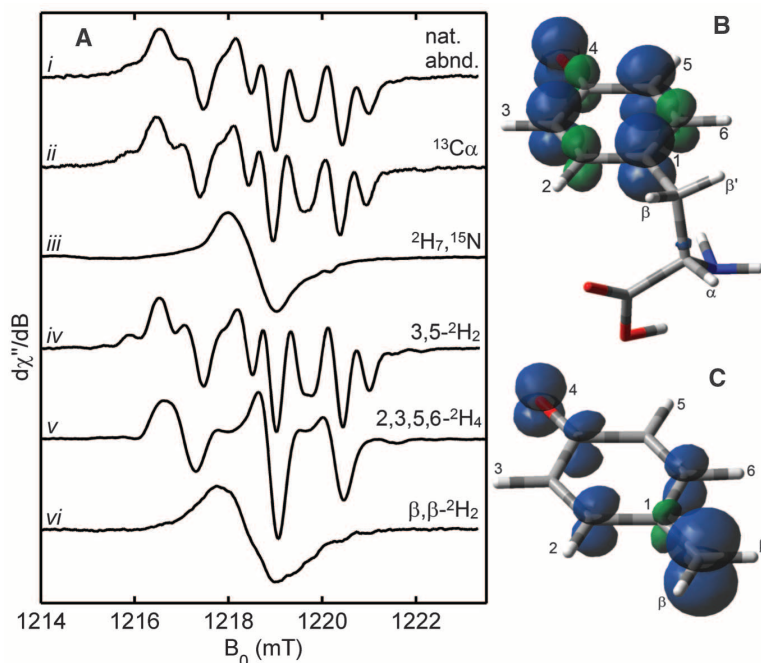


Fig. 4. Continuous-wave EPR spectra of the freeze-quenched HydG^{WT} reaction with specific Tyr isotopologs, along with corresponding DFT-based electronic structures. (A) Q-band (34.17 GHz, 112 K, 9.6 μW) EPR spectra of 30-s reaction samples with specific Tyr isotopologs. (i) Natural abundance Tyr, (ii) $^{13}\text{C}_\alpha$ -Tyr, (iii) $^2\text{H}_7$, ^{15}N -Tyr, (iv) $3,5\text{-}^2\text{H}_2$ -Tyr, (v) $2,3,5,6\text{-}^2\text{H}_4$ -Tyr, and (vi) $\beta, \beta\text{-}^2\text{H}_2$ -Tyr. (B) DFT-derived spin density of a neutral $\text{Tyr}^\bullet$ radical. (C) DFT-derived spin density of the observed $4\text{OB}^\bullet$ radical.

some secondary path, such as oxidative decarboxylation of DHG (30).

Our proposed mechanism begins with SAM bound at the N-terminal cluster and tyrosine bound at the C-terminal cluster (Fig. 3, step I), with reductive cleavage of SAM generating 5'-dA[•] (Fig. 3, step II). This radical is quenched by the abstraction of a solvent-exchangeable H atom (see mass spectrometric data, fig. S5), consistent with the proposal (20) that 5'-dA[•] abstracts the phenolic H of free tyrosine. The resulting Tyr[•] radical is ligated to the C-terminal 4Fe-4S cluster (Fig. 3, step III) and is not currently observed in the RFQ EPR experiments. The specific coordination geometry of the Tyr[•] bound to the C-terminal 4Fe-4S cluster may play a direct role in directing the subsequent C_α-C_β bond cleavage along the heterolytic pathway, concomitantly forming the observed 4OB[•] and DHG ligated to the 4Fe-4S cluster (Fig. 3, step IV). Free DHG is unstable and rapidly hydrolyzes to produce glyoxylate and ammonia, observed as by-products in the ThiH reaction in the absence of the other required thiazole precursors (19). In the case of HydG, scission of the 4Fe-4S-bound DHG occurs to yield Fe-bound CO and CN⁻, concomitant with electron and proton transfer to 4OB[•] to form the *p*-cresol product of the HydG reaction (Fig. 3, step V). Given the facile interconversion between 4Fe-4S and 3Fe-4S forms observed in proteins such as aconitase (22, 23, 31) and ferredoxins (32), this unique CO- and CN⁻-loaded Fe may then be inserted into the assembly of the 2Fe subunit of

the H cluster. We are currently using a variety of spectroscopy techniques to reveal further details of this fascinating metallocofactor assembly process.

References and Notes

- P. M. Vignais, B. Billoud, *Chem. Rev.* **107**, 4206–4272 (2007).
- K. A. Vincent, A. Parkin, F. A. Armstrong, *Chem. Rev.* **107**, 4366–4413 (2007).
- J. W. Peters, W. N. Lanzilotta, B. J. Lemon, L. C. Seefeldt, *Science* **282**, 1853–1858 (1998).
- A. S. Pandey, T. V. Harris, L. J. Giles, J. W. Peters, R. K. Szilagyi, *J. Am. Chem. Soc.* **130**, 4533–4540 (2008).
- G. Berggren *et al.*, *Nature* **499**, 66–69 (2013).
- J. Esselborn *et al.*, *Nat. Chem. Biol.* **9**, 607–609 (2013).
- D. W. Mulder *et al.*, *Structure* **19**, 1038–1052 (2011).
- P. A. Frey, A. D. Hegeman, F. J. Ruzicka, *Crit. Rev. Biochem. Mol. Biol.* **43**, 63–88 (2008).
- J. L. Vey, C. L. Drennan, *Chem. Rev.* **111**, 2487–2506 (2011).
- http://sfld.rvbi.ucsf.edu/django/superfamily/29/.
- E. Pilet *et al.*, *FEBS Lett.* **583**, 506–511 (2009).
- R. C. Driesener *et al.*, *Angew. Chem. Int. Ed.* **49**, 1687–1690 (2010).
- E. M. Shepard *et al.*, *J. Am. Chem. Soc.* **132**, 9247–9249 (2010).
- J. M. Kuchenreuther, S. J. George, C. S. Grady-Smith, S. P. Cramer, J. R. Swartz, *PLOS ONE* **6**, e20346 (2011).
- J. K. Rubach, X. Brazzolotto, J. Gaillard, M. Fontecave, *FEBS Lett.* **579**, 5055–5060 (2005).
- C. Tron *et al.*, *Eur. J. Inorg. Chem.* **2011**, 1121–1127 (2011).
- Y. Nicolet, J. C. Fontecilla-Camps, *J. Biol. Chem.* **287**, 13532–13540 (2012).
- M. Kriek *et al.*, *J. Biol. Chem.* **282**, 17413–17423 (2007).
- M. Kriek, F. Martins, M. R. Challand, A. Croft, P. L. Roach, *Angew. Chem. Int. Ed.* **46**, 9223–9226 (2007).
- Y. Nicolet, L. Martin, C. Tron, J. C. Fontecilla-Camps, *FEBS Lett.* **584**, 4197–4202 (2010).
- J. M. Kuchenreuther, R. D. Britt, J. R. Swartz, *PLOS ONE* **7**, e45850 (2012).
- H. Beinert, M. C. Kennedy, C. D. Stout, *Chem. Rev.* **96**, 2335–2374 (1996).
- M. C. Kennedy *et al.*, *J. Biol. Chem.* **259**, 14463–14471 (1984).
- A. J. M. Richards, A. J. Thomson, R. H. Holm, K. S. Hagen, *Spectrochim. Acta Part A Molec. Biomolec. Spectrosc.* **46**, 987–993 (1990).
- C. J. Fugate *et al.*, *J. Am. Chem. Soc.* **134**, 9042–9045 (2012).
- B. A. Barry, G. T. Babcock, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 7099–7103 (1987).
- M. L. Gilchrist Jr., J. A. Ball, D. W. Randall, R. D. Britt, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 9545–9549 (1995).
- R. J. Hulsebosch *et al.*, *J. Am. Chem. Soc.* **119**, 8685–8694 (1997).
- F. Dole, B. A. Diner, C. W. Hoganson, G. T. Babcock, R. D. Britt, *J. Am. Chem. Soc.* **119**, 11540–11541 (1997).
- R. Michaels, L. V. Hanks, W. A. Corpe, *Arch. Biochem. Biophys.* **111**, 121–125 (1965).
- T. A. Kent *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 1096–1100 (1982).
- S. J. George, F. A. Armstrong, E. C. Hatchikian, A. J. Thomson, *Biochem. J.* **264**, 275–284 (1989).

Acknowledgments: This work was funded by the Division of Chemical Sciences, Geosciences, and Biosciences (R.D.B. award no. DE-FG02-11ER16282) and the Division of Material Sciences and Engineering (J.R.S. award no. DE-FG02-09ER46632) of the Office of Basic Energy Sciences of the U.S. Department of Energy. We thank D. Suesse for useful discussions on the proposed HydG mechanism.

Supplementary Materials

www.sciencemag.org/content/342/6157/472/suppl/DC1
Materials and Methods
Figs. S1 to S5
Table S1
References (33–48)

13 June 2013; accepted 20 September 2013
10.1126/science.1241859

Causes and Effects of N-Terminal Codon Bias in Bacterial Genes

Daniel B. Goodman,^{1,2,3} George M. Church,^{1,2*} Sriram Kosuri^{1*}

Most amino acids are encoded by multiple codons, and codon choice has strong effects on protein expression. Rare codons are enriched at the N terminus of genes in most organisms, although the causes and effects of this bias are unclear. Here, we measure expression from >14,000 synthetic reporters in *Escherichia coli* and show that using N-terminal rare codons instead of common ones increases expression by ~14-fold (median 4-fold). We quantify how individual N-terminal codons affect expression and show that these effects shape the sequence of natural genes. Finally, we demonstrate that reduced RNA structure and not codon rarity itself is responsible for expression increases. Our observations resolve controversies over the roles of N-terminal codon bias and suggest a straightforward method for optimizing heterologous gene expression in bacteria.

Codon usage is biased in natural genes and can strongly affect heterologous expression (1). Many organisms are enriched for poorly adapted codons at the N terminus of genes (2–5). Several studies suggest that these codons slow ribosomal elongation during initiation and lead to

increased translational efficiency (2, 4, 6). Most organisms also display reduced mRNA secondary structure at the N terminus (7), and studies using synthetic codon gene variants have resulted in conflicting theories on which mechanisms are causal for expression changes (7, 8). Information about the causes and effects of codon bias has been restricted to relations inferred from natural sequences using genome-wide correlation (2, 3, 5, 9, 10), conservation among species (4), or relatively small libraries of synthetic genes with synonymous codon changes (3, 8, 11–15). Here, we separate and quantify the factors controlling expression at the N terminus

of genes in *Escherichia coli* by building and measuring expression from a large synthetic library of defined sequences.

We used array-based oligonucleotide libraries (16) to generate 14,234 combinations of promoters, ribosome binding sites (RBSs), and 11 N-terminal codons in front of super-folder green fluorescent protein (sfGFP) on a plasmid that constitutively coexpresses mCherry (fig. S1) (17–19). The sequences for the N-terminal peptides correspond to the first 11 amino acids (including the initiating methionine) of 137 endogenous *E. coli* essential genes (20) that utilize the entire codon repertoire (fig. S2). We expressed these sfGFP fusions from two promoters and three RBSs of varying strengths (19). We also included the natural RBS for each endogenous gene. For each combination of promoter, RBS, and peptide sequence, we designed a set of 13 codon variants to represent a wide range of codon usages and secondary structure free energies across the translation initiation region. We studied the interactions between the 5' untranslated region (UTR) and N-terminal codon usage because initiation is thought to be the rate-limiting step for translation (1), this region has been previously implicated in determining most expression variation (8), N-terminal codons are more highly conserved (21), and rare codons are enriched at the N terminus of natural genes and especially those that are highly expressed (2).

¹Wyss Institute for Biologically Inspired Engineering, 3 Blackfan Circle, Boston, MA 02115, USA. ²Department of Genetics, Harvard Medical School, 77 Avenue Louis Pasteur, Boston, MA 02115, USA. ³Harvard-MIT Health Sciences and Technology, 77 Massachusetts Avenue, Cambridge, MA 02139, USA.

*Corresponding author. E-mail: sri.kosuri@wyss.harvard.edu (S.K.); gchurch@genetics.med.harvard.edu (G.M.C.)

We measured DNA, RNA, and protein levels from the entire library using a multiplex assay (Fig. 1C and figs. S3 and S4) (19). DNA and RNA levels were determined using DNA sequencing (DNaseq) and RNAseq. Protein levels were determined by FlowSeq; 7327 (51.5%) constructs were within the quantitative range of our assay [coefficient of determination (R^2) = 0.955, $P < 2 \times$

10^{-16}] (fig. S5). We normalized the expression measurements across each 13-member codon variant set as fold change from log-average to control for changes in promoters, RBSs, and peptide sequence (fig. S6). Changing synonymous codon usage in the 11-amino acid N-terminal peptide resulted in a mean 60-fold increase in protein abundance from

the weakest to strongest codon variant even though >96% of the gene remained unchanged. For over 160 codon variant sets (25% of sets within range), the difference was >100-fold. For each codon variant set, we included sequences encoding the most common or rare synonymous codon in *E. coli* for every amino acid. The rare codon constructs displayed a mean 14-fold (median 4-fold)

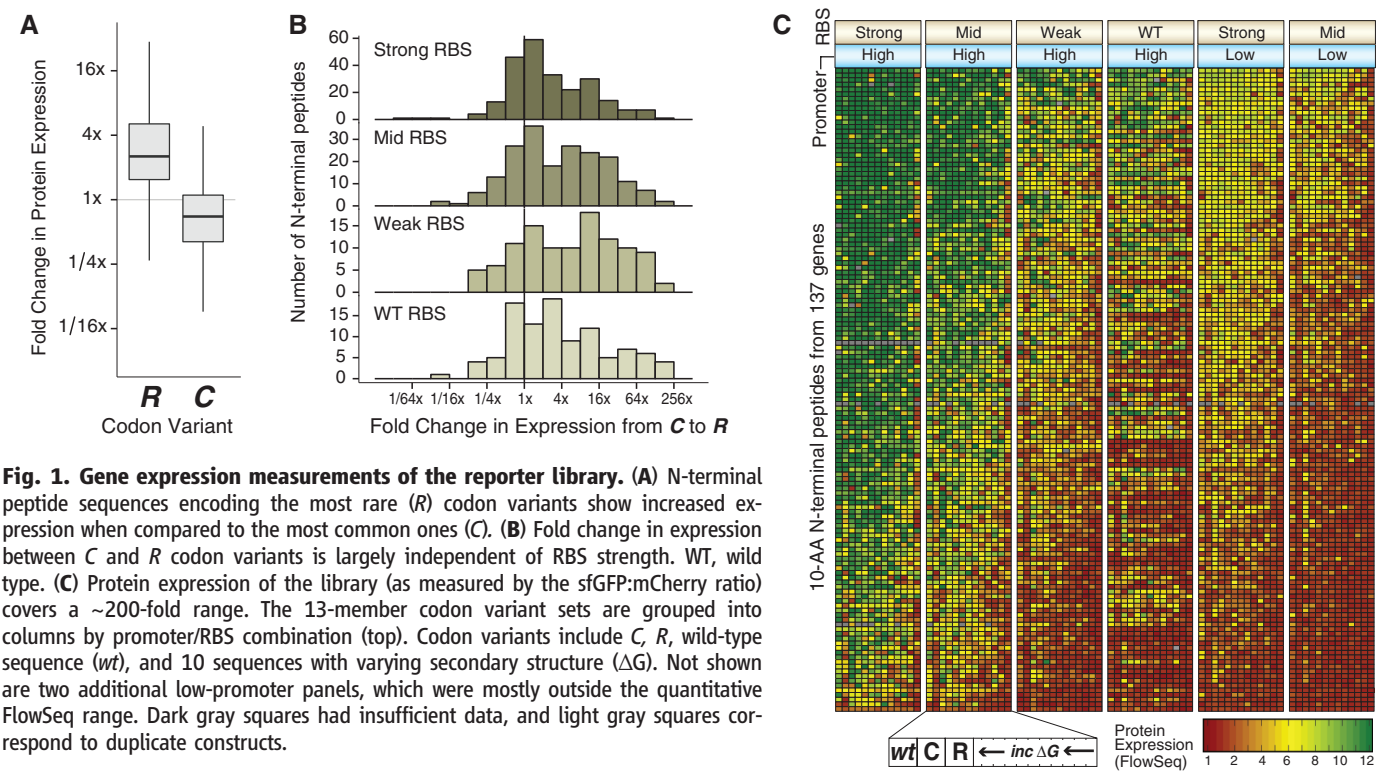


Fig. 1. Gene expression measurements of the reporter library. (A) N-terminal peptide sequences encoding the most rare (*R*) codon variants show increased expression when compared to the most common ones (*C*). (B) Fold change in expression between *C* and *R* codon variants is largely independent of RBS strength. WT, wild type. (C) Protein expression of the library (as measured by the sfGFP:mCherry ratio) covers a ~200-fold range. The 13-member codon variant sets are grouped into columns by promoter/RBS combination (top). Codon variants include *C*, *R*, wild-type sequence (*wt*), and 10 sequences with varying secondary structure (ΔG). Not shown are two additional low-promoter panels, which were mostly outside the quantitative FlowSeq range. Dark gray squares had insufficient data, and light gray squares correspond to duplicate constructs.

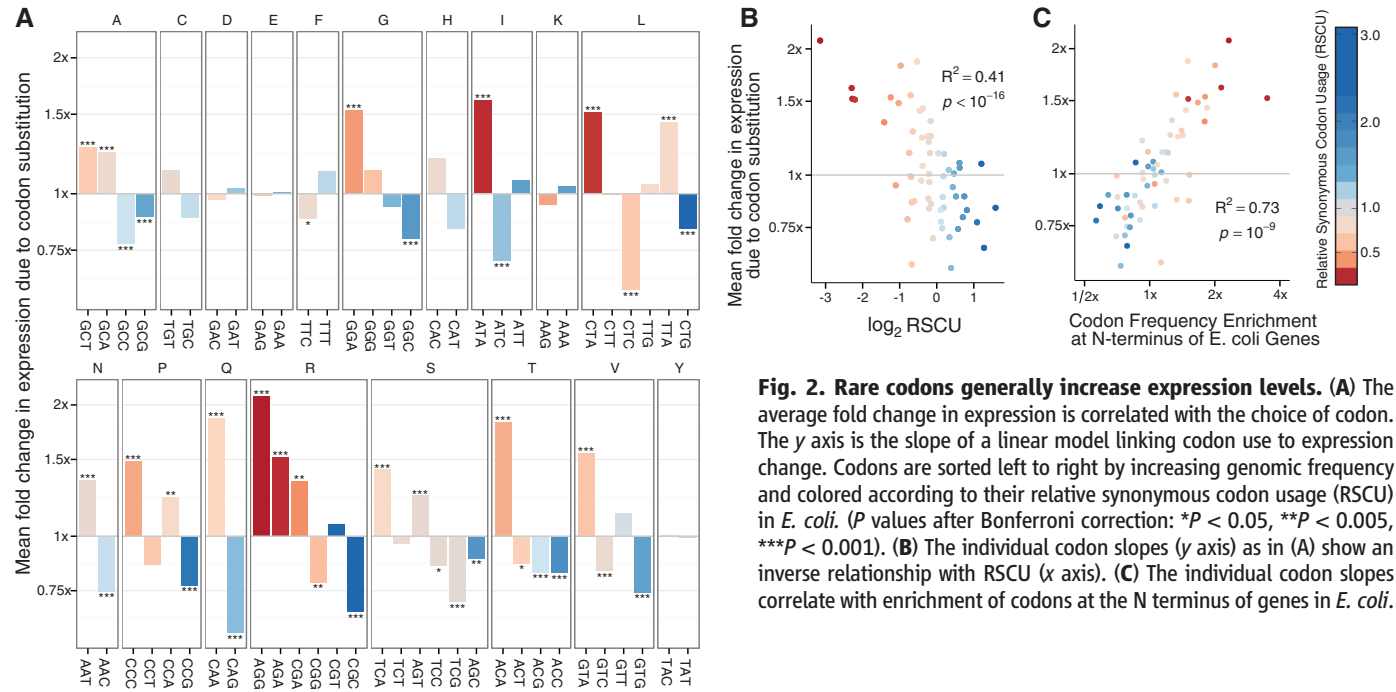


Fig. 2. Rare codons generally increase expression levels. (A) The average fold change in expression is correlated with the choice of codon. The y axis is the slope of a linear model linking codon use to expression change. Codons are sorted left to right by increasing genomic frequency and colored according to their relative synonymous codon usage (RSCU) in *E. coli*. (P values after Bonferroni correction: $*P < 0.05$, $**P < 0.005$, $***P < 0.001$). (B) The individual codon slopes (y axis) as in (A) show an inverse relationship with RSCU (x axis). (C) The individual codon slopes correlate with enrichment of codons at the N terminus of genes in *E. coli*.

increase in protein abundance compared with common codon constructs (Fig. 1A) ($P < 2 \times 10^{-16}$, two-tailed t test) even though common codons are generally thought to increase protein expression and fitness (1, 9, 22, 23).

To understand why rare codons cause increased expression, we first examined several codon usage metrics, but they could only explain $<5\%$ of expression differences (fig. S7A). New metrics that take into account both transfer RNA (tRNA) availability and usage [normalized translational efficiency (nTE)] show stronger N-terminal enrichment (4). We calculated nTE scores for *E. coli* and found that nTE scores that were similar to the tRNA adaptation index (tAI) ($R^2 = 0.847$, $P < 2 \times 10^{-16}$) did not correlate well with N-terminal codon enrichment in the *E. coli* genome ($R^2 = 0.107$, $P = 0.00654$), and did not significantly correlate with codons that increased protein expression in our data set ($R^2 = 0.024$, $P = 0.124$). Others have proposed that slow ribosome progression at the N

terminus due to rare codons increases translational efficiency (2, 13, 14). This “codon ramp” hypothesis should apply primarily in the context of strong translation, but we found that using rare codons at the N terminus increases expression regardless of translation strength (Fig. 1B). Finally, ribosome occupancy profiling in *E. coli* has shown that tRNA abundance does not correlate to translation rate but that specific rare codons can create internal Shine-Dalgarno-like motifs that can alter translational efficiency (6). We looked for an association between the presence of internal Shine-Dalgarno-like motifs and changes in expression, and found it to be weak but statistically significant ($R^2 = 0.002$, $P < 1.3 \times 10^{-5}$).

We built a simple linear regression model correlating the use of each individual synonymous codon with expression changes (Fig. 2A and fig. S8). For most amino acids, we found a link between the rarity of the codon and increased expression (Fig. 2B). There is a strong correlation

between codons that affected expression and their relative N-terminal enrichment in *E. coli* ($R^2 = 0.73$, $P < 2.3 \times 10^{-9}$) (Fig. 2C). Using relative translation efficiency instead of relative expression produced similar results (fig. S9).

Decreased GC-content correlated with increased protein expression ($R^2 = 0.12$, $P < 2 \times 10^{-16}$) (Fig. 3A). Rare codons in *E. coli* are frequently A/T-rich at the third position, and codons ending in A/T more frequently correlate with increased expression than synonymous codons ending in G/C. (fig. S10). This association suggested a link to mRNA transcript secondary structure (8), and so we computationally predicted RNA structure over the first 120 bases of each transcript using NUPACK (24). We found that increased secondary structure was correlated with decreased expression, which explained more variation than any other variable we measured ($R^2 = 0.34$, $P < 2 \times 10^{-16}$) (Fig. 3B). We made a similar linear regression model relating individual codon substitution

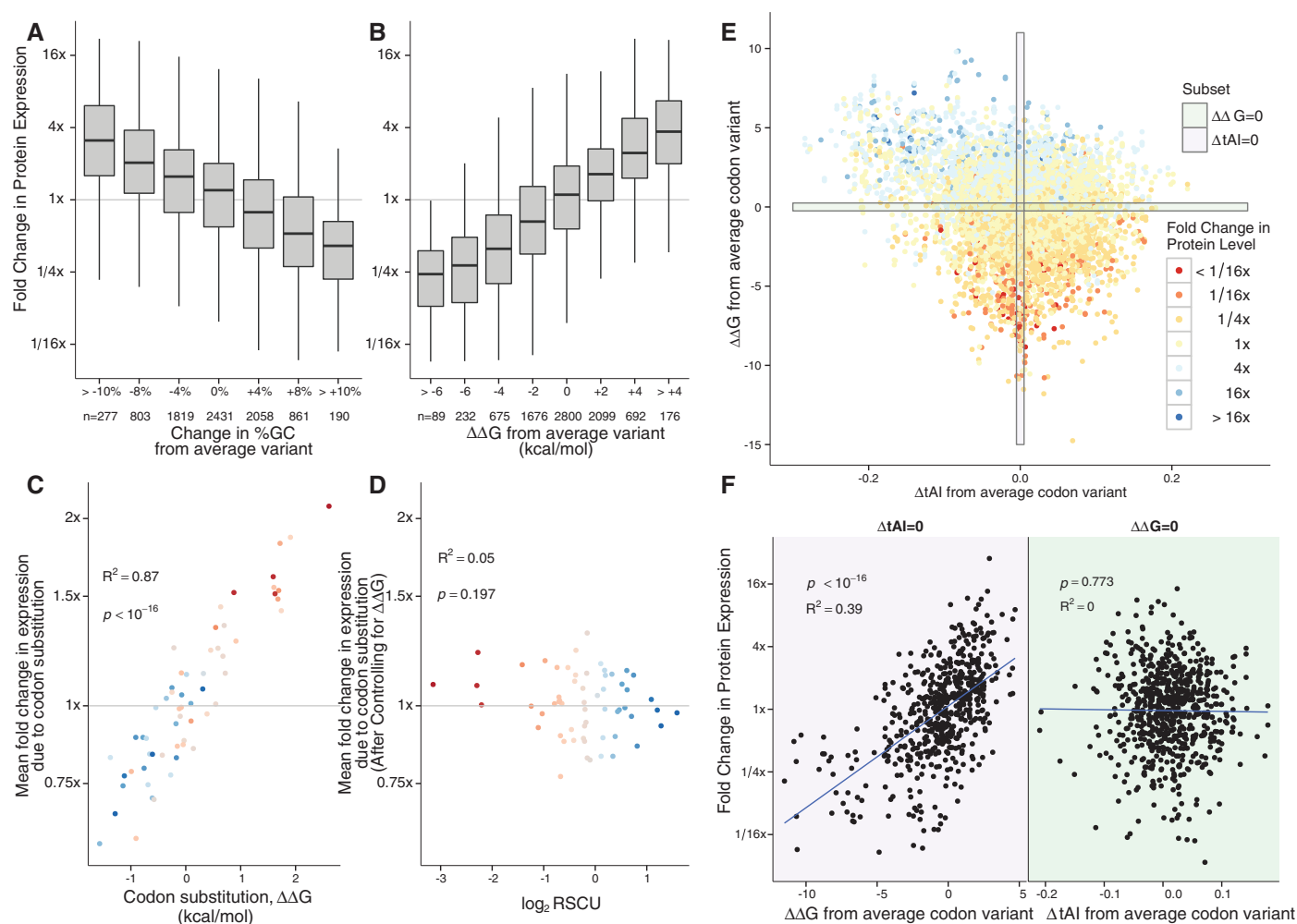


Fig. 3. Rare codons alter expression by reducing mRNA secondary structure. (A) Expression changes are correlated with relative changes in %GC content. Each boxplot includes $\pm 2\%$ of centered value. (B) Expression increases correlate to relative increases in free energy of folding at the front of the transcript ($\Delta\Delta G$). Each boxplot includes ± 2 kcal/mol of centered value. (C) Individual codon slopes (same as Fig. 2A y axis) correlate with the $\Delta\Delta G$ per individual codon substitution. (D) After controlling for $\Delta\Delta G$ with a multiple

linear regression, there is no longer any relation between individual codon slopes and RSCU (compare with Fig. 2B). (E) The $\Delta\Delta G$ versus change in tAI is plotted for all constructs within the quantitative range. Constructs are colored by their relative fold change in expression from the average codon variant within the set. (F) Subsets of constructs corresponding to the shaded boxes in (E). (Left) Points with constant codon adaptation and varied secondary structure, (right) points with constant secondary structure and varied codon adaptation.

to change in secondary structure free energy, rather than expression levels, and found a strong correlation between codons that decreased secondary structure and those that increased protein expression ($R^2 = 0.87$, $P < 2 \times 10^{-16}$) (Fig. 3C). In addition, codon adaptation metrics at the N terminus correlate as well to change in secondary structure free energy as they do to change in protein expression (fig. S7B).

We used multiple regression to control for the secondary structure changes between codon variants and found that no relation remained between N-terminal codon adaptation and increased expression ($R^2 = 0.05$, $P = 0.197$) (Fig. 3D). Additionally, constructs with constant tAI still show a correlation between expression and secondary structure, but constructs with constant secondary structure have no correlation between tAI and expression. (Fig. 3, E and F). Finally, if secondary structure is the dominant factor, we would expect a disproportionate enrichment of A over T due to G-U wobble pairing. Indeed, nucleotide triplets with A at the wobble position were more consistently correlated with expression in our data set and with enrichment at the N terminus of *E. coli* genes (fig. S11).

Kudla *et al.* show that local RNA structure in the region between -4 and +38 of translation start

is most correlated with expression change (8). Our data indicate that the region centered on +10 is most correlated with expression changes (Fig. 4 and figs. S12 to S14), which closely matches in vitro translation studies (25). This region remained the most correlated for the subset of constructs with no change in total free energy of folding across the N-terminal region (figs. S15 and S16). Although secondary structure is known to affect the RBS (26), when only codon usage is altered, RNA structure after the start codon, and not at the RBS, is the major contributor to expression differences. A multiple linear regression model that combines promoter and RBS choice, as well as N-terminal secondary structure and GC content, still explains only 54% of variation in expression levels. Amino acid composition effects on sfGFP folding and inadequacies in computational RNA structure prediction could be partially responsible. However, there are likely additional effects left to uncover, and the extent to which codon usage beyond the N-terminal region alters gene expression remains unresolved (8, 14).

The N terminus of genes in almost all bacteria displays reduced secondary structure, but enrichment of poorly adapted N-terminal codons is only found in bacteria with GC content of at least 50% (3). Recent work further shows that AT-rich

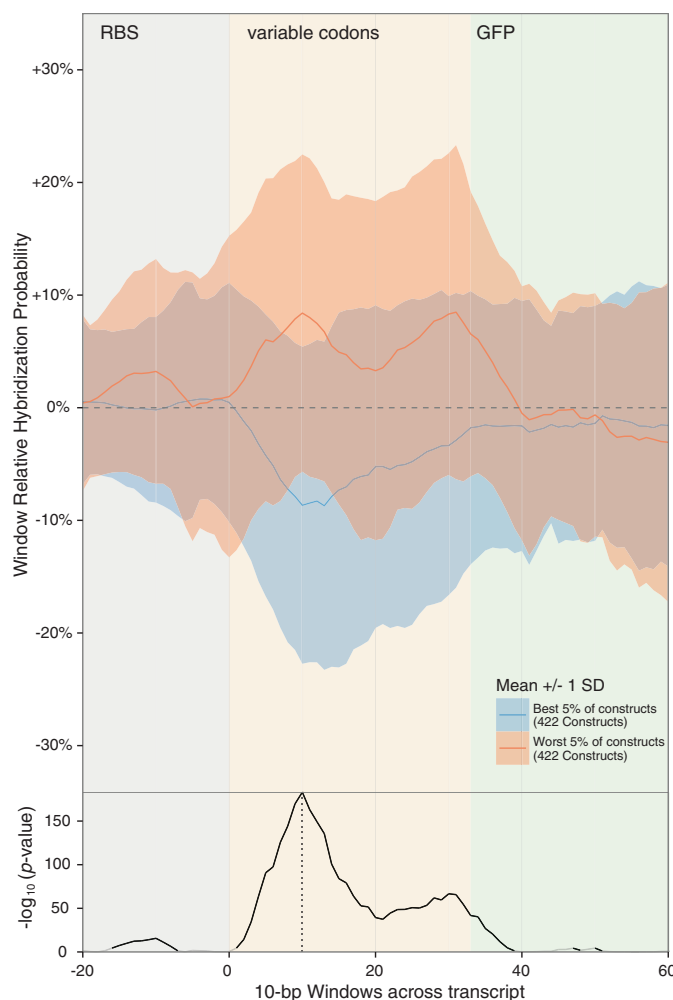
codons as opposed to rare codons themselves are preferentially selected, which implicates secondary structure as the driving force for N-terminal codon selection in most bacteria (5). Despite mechanistic differences in translation between prokaryotes and eukaryotes, both single- and multicell eukaryotes also have reduced N-terminal secondary structure (7). For synthetic GFP templates in yeast, secondary structure is more correlated with expression changes than codon adaptation metrics (10). Here, we do not examine other factors that might shape natural sequence, such as codon pair bias (1, 27), cotranslational folding (4, 12, 28), or growth conditions (11, 15). Natural genomic sequence is often not suited to distinguish between conflicting hypotheses of how sequence affects function; multiplexed assays of large synthetic DNA libraries provide a powerful method to examine such hypotheses in a controlled manner.

References and Notes

1. J. B. Plotkin, G. Kudla, *Nat. Rev. Genet.* **12**, 32–42 (2011).
2. T. Tuller *et al.*, *Cell* **141**, 344–354 (2010).
3. M. Allert, J. C. Cox, H. W. Hellenga, *J. Mol. Biol.* **402**, 905–918 (2010).
4. S. Pechmann, J. Frydman, *Nat. Struct. Mol. Biol.* **20**, 237–243 (2013).
5. K. Bentele, P. Saffert, R. Rauscher, Z. Ignatova, N. Blüthgen, *Mol. Syst. Biol.* **9**, 675 (2013).
6. G.-W. Li, E. Oh, J. S. Weissman, *Nature* **484**, 538–541 (2012).
7. W. Gu, T. Zhou, C. O. Wilke, *PLOS Comput. Biol.* **6**, e1000664 (2010).
8. G. Kudla, A. W. Murray, D. Tollervey, J. B. Plotkin, *Science* **324**, 255–258 (2009).
9. M. dos Reis, R. Savva, L. Wernisch, *Nucleic Acids Res.* **32**, 5036–5044 (2004).
10. P. Shah, Y. Ding, M. Niemczyk, G. Kudla, J. B. Plotkin, *Cell* **153**, 1589–1601 (2013).
11. M. Welch *et al.*, *PLOS ONE* **4**, e7002 (2009).
12. M. Zhou *et al.*, *Nature* **495**, 111–115 (2013).
13. S. Navon, Y. Pilpel, *Genome Biol.* **12**, R12 (2011).
14. T. Tuller, Y. Y. Waldman, M. Kupiec, E. Ruppin, *Proc. Natl. Acad. Sci. U.S.A.* **107**, 3645–3650 (2010).
15. A. R. Subramaniam, T. Pan, P. Cluzel, *Proc. Natl. Acad. Sci. U.S.A.* **110**, 2419–2424 (2013).
16. E. M. LeProust *et al.*, *Nucleic Acids Res.* **38**, 2522–2540 (2010).
17. J.-D. Pédélec, S. E. P. Cabantous, T. Tran, T. C. Terwilliger, G. S. Waldo, *Nat. Biotechnol.* **24**, 79–88 (2006).
18. N. C. Shaner *et al.*, *Nat. Biotechnol.* **22**, 1567–1572 (2004).
19. S. Kosuri *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **110**, 14024–14029 (2013).
20. Y. Yamazaki, H. Niki, J.-I. Kato, *Methods Mol. Biol.* **416**, 385–389 (2008).
21. D. L. Hartl, E. N. Moriyama, S. A. Sawyer, *Genetics* **138**, 227–234 (1994).
22. M. Gouy, C. Gautier, *Nucleic Acids Res.* **10**, 7055–7074 (1982).
23. P. M. Sharp, W. H. Li, *Nucleic Acids Res.* **15**, 1281–1295 (1987).
24. J. N. Zadeh *et al.*, *J. Comput. Chem.* **32**, 170–173 (2011).
25. D. Voges, M. Watzel, C. Nemetz, S. Witzemann, B. Buchberger, *Biochem. Biophys. Res. Commun.* **318**, 601–614 (2004).
26. M. H. de Smit, J. van Duin, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 7668–7672 (1990).
27. J. R. Coleman *et al.*, *Science* **320**, 1784–1787 (2008).
28. A. A. Komar, *Trends Biochem. Sci.* **34**, 16–24 (2009).

Acknowledgments: We thank J. C. Way, E. R. Daugherty, and R. T. Sauer for comments. The research was supported by the U.S. Department of Energy (DE-FG02-02ER63445 to G.M.C.), NSF SynBERC (SAS283-11210 to G.M.C.), Office of Naval Research (N000141010144 to G.M.C. and S.K.), Agilent

Fig. 4. mRNA structure downstream of start codon is most correlated with reduced expression. Relative hybridization probabilities averaged in 10-nucleotide windows are plotted against their correlation with expression change as a function of position (–20 to +60 from ATG). (Top) The best and worst 5% of constructs—as ranked by relative expression within a codon variant set—are grouped and plotted as blue and red ribbons, respectively. The ribbon tops and bottoms are one standard deviation from the mean, which is shown as a solid line. (Bottom) The P value for linear regressions, correlating hybridization probabilities within each window to expression fold change in all constructs.



Technologies, Wyss Institute, and an NSF Graduate Research Fellowship to D.B.G. Data can be accessed on the National Center for Biotechnology Information, NIH, Sequence Read Archive (SRA) (SRP029609). pGERC reporter can be obtained from AddGene (#47441). Accession numbers: The Project accession at the SRA is SRP029609. The sample accession is SRS477429. There are three experiments, one for DNA, one for

RNA, one for FlowSeq: RNA, SRX346948; DNA, SRX346944; and FlowSeq, SRX346268.

Supplementary Materials

www.sciencemag.org/content/342/6157/475/suppl/DC1
Materials and Methods
Supplementary Text

Figs. S1 to S16
Table S1
References (29–35)

14 June 2013; accepted 13 September 2013
Published online 26 September 2013;
10.1126/science.1241934

2000 Years of Parallel Societies in Stone Age Central Europe

Ruth Bollongino,^{1*} Olaf Nehlich,^{2,3} Michael P. Richards,^{2,3,4} Jörg Orschiedt,⁵ Mark G. Thomas,⁶ Christian Sell,¹ Zuzana Fajkošová,¹ Adam Powell,¹ Joachim Burger¹

Debate on the ancestry of Europeans centers on the interplay between Mesolithic foragers and Neolithic farmers. Foragers are generally believed to have disappeared shortly after the arrival of agriculture. To investigate the relation between foragers and farmers, we examined Mesolithic and Neolithic samples from the Blätterhöhle site. Mesolithic mitochondrial DNA sequences were typical of European foragers, whereas the Neolithic sample included additional lineages that are associated with early farmers. However, isotope analyses separate the Neolithic sample into two groups: one with an agriculturalist diet and one with a forager and freshwater fish diet, the latter carrying mitochondrial DNA sequences typical of Mesolithic hunter-gatherers. This indicates that the descendants of Mesolithic people maintained a foraging lifestyle in Central Europe for more than 2000 years after the arrival of farming societies.

The Mesolithic-Neolithic transition marks a shift from a foraging to an agricultural way of life. It first appeared around 8500 BC in present-day southeastern Anatolia and Syria. About 3000 years later, this subsistence strategy reached Central Europe through the expansion of the Neolithic Linear Pottery culture (LBK). Whether the first European farmers descended from hunter-gatherers or migrated in from the Near East has been debated extensively in the archaeological literature. Over the last decade, a number of palaeogenetic studies have contributed substantially to current understanding of the Mesolithic-Neolithic transition in Europe [(1) and references therein]. Taken together, these findings strongly support a demic diffusion of early farmers into Central Europe, most likely originating in the southeast of the continent (2). Little is known about how long hunter-gatherers persisted in Central Europe, as there are no unambiguous signs of their presence in the archaeological record after the Early Neolithic. In this study, we present both ancient DNA and isotopic data, which, when combined, provide persuasive evidence for the prolonged coexistence of genetically distinct hunter-gatherer and farming groups over the course of the Neolithic in Central Europe.

Ancient DNA and sulfur, nitrogen, and carbon isotope ratios were analyzed from bones and teeth of 29 individuals from a burial cave site that contained around 450 remains from both Meso-

lithic hunter-gatherers and Neolithic individuals. The Blätterhöhle site is situated in Hagen, Germany (3) (Fig. 1), and because of its long and narrow geological structure, it is very likely that the human remains were deposited deliberately. Because the layers inside the cave have been disturbed by bioturbation, all samples used in this study were ¹⁴C dated by accelerator mass spectrometry. The ¹⁴C dates reveal two occupation phases ranging from 9210 to 8340 calibrated BCE (cal BC) (Mesolithic) and from 3986 to 2918 cal BC (Neolithic), respectively (4) (Table 1, table S1, and fig. S1).

We applied both a polymerase chain reaction, with subsequent Sanger sequencing, and a capture next-generation sequencing approach to establish partial or complete mitochondrial genomes. Out of 29 samples, 25 yielded reproducible mitochondrial hypervariable region I (HVRI) sequences (4) (Table 1 and tables S4 and S6). Complete mitochondrial genomes with coverage from 3.6× up to 39.8× were obtained for one Mesolithic and five Neolithic samples (4) (tables S4 and S6).

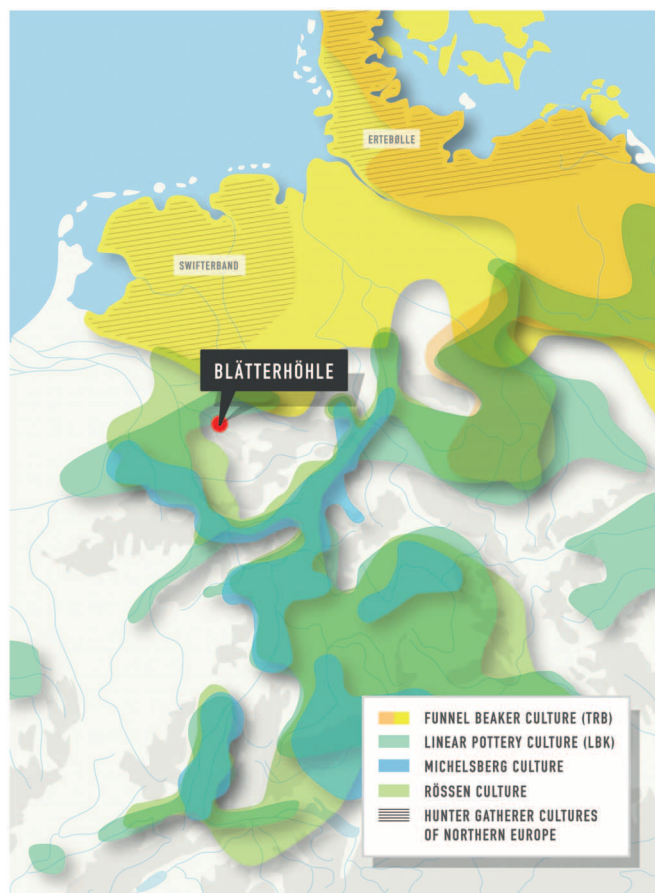


Fig. 1. Geographic location of the Blätterhöhle cave site with schematic representation of the distribution of relevant archaeological cultures in Central and Northern Europe (27).

¹Palaeogenetics Group, Institute of Anthropology, Johannes Gutenberg University, 55099 Mainz, Germany. ²Department of Anthropology, University of British Columbia, Vancouver, British Columbia V6T 1Z1, Canada. ³Department of Human Evolution, Max Planck Institute for Evolutionary Anthropology, Deutscher Platz 6, 04103 Leipzig, Germany. ⁴Department of Archaeology, University of Durham, Durham, DH1 3LE, UK. ⁵Institute of Prehistoric Archaeology, Free University of Berlin, 14195 Berlin, Germany. ⁶Research Department of Genetics, Evolution and Environment, University College London, Gower Street, London WC1E 6BT, UK.

*Corresponding author. E-mail: bollongi@uni-mainz.de

All five Mesolithic samples belong to the mitochondrial haplogroup (hg) U, in common with all previously typed pre-Neolithic hunter-gatherers of Central, Eastern, and Northern Europe (2, 5). Twelve of the Neolithic sequences also belong to hg U, whereas eight belong to other mitochondrial clades, such as hg H and J, as typically observed in early Neolithic farmers (1). Although U lineages are rare among early farmers, they appear at an unexpectedly high frequency [60%: 95% confidence interval (CI) = 38 to 78%] in our Late Neolithic Blätterhöhle sample. So at first glance, the Neolithic population of Blätterhöhle would appear to be a group of farmers with a high proportion of Mesolithic ancestry. However, dietary stable isotope evidence from the same specimens reveals a very different picture.

Three main clusters can be identified from the carbon and nitrogen isotopes plot (Fig. 2 and table S7): A first group, comprising all Mesolithic individuals, has particularly low $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. This is consistent with a diet primarily of the wild fauna identified in the cave and similar to other inland Mesolithic sites (6, 7). The second group is from the Late Neolithic, and its isotope values reflect a terrestrial diet of herbivores, most likely domesticated animals. Their $\delta^{15}\text{N}$ values are slightly enriched in ^{15}N compared with the Mesolithic group, which may be explained by the cultural practice of manuring the pasture land where domesticated animals were raised (8). This kind of enriched $\delta^{15}\text{N}$ is also observed in other Neolithic farming sites in Germany (9, 10). The third group also dates to the Late Neolithic, but, in contrast to groups 1 and 2, the isotope results point to a highly unusual diet, at least for the Neolithic period. In this group both carbon and nitrogen isotope values are very enriched, which suggests a diet low in plant and herbivore protein and high in freshwater fish (11, 12). The $\delta^{34}\text{S}$ values strengthen this interpretation by showing their dietary protein came from freshwater sources (12) (Fig. 2B), whereas the Mesolithic hunter-gatherers and Neolithic farmers consumed terrestrial plants and animals.

Like the Mesolithic individuals of group 1, the mitochondrial DNA (mtDNA) sequences of the Neolithic freshwater fish consumers consist solely of U lineages, with none of the mitochondrial lineages typically observed in farmers (2). Thus, group 3 not only had a fisher-hunter-gatherer diet and lifestyle but also a signature of European Mesolithic hunter-gatherer ancestry. In contrast, the contemporaneous group 2 carried both mitochondrial lineages typically found in European Neolithic farmers and those found in European Mesolithic hunter-gatherers. As their isotope signature shows the typical pattern of individuals that consume domestic herbivores, they can be considered agriculturalists.

In summary, the results of ^{14}C and stable isotope analysis, together with the DNA evidence, suggest that the Blätterhöhle individuals are sampled from three distinct populations: (i) Mesolithic hunter-gatherers, (ii) Neolithic farmers, and (iii) Neolithic fisher-hunter-gatherers (specializing in freshwater fish). The latter two notably date to the fourth mil-

lennium BC, which is around 2000 years after the introduction of farming to Central Europe.

Genetically, Blätterhöhle Neolithic fisher-hunter-gatherers appear rather similar to other Neolithic hunter-gatherers (table S9C) but exhibit a relatively high (albeit not significant) fixation index (or proportion of genetic variation due to population differentiation, F_{ST}) value of 0.107 compared with the Mesolithic hunter-gatherers from the same cave site 5000 years before. At first sight, this would seem to cast doubt on a direct genetic continuity between them. However, coalescent simulations do not reject a model of population continuity under a very wide range of demographic parameters (4) (fig. S6A).

In another demographic model, we tested for continuity between Late Neolithic farmers and modern Europeans ($F_{\text{ST}} = 0.016$) (table S9B). Although Bramanti *et al.* (2) found that population continuity between early (LBK) farmers and modern Europeans could be rejected, our coalescent simulations provide no evidence to reject continuity between the later Neolithic farmers (table S8) and modern Europeans (4) (table S8 and fig. S6B). This indicates a pivotal role for admixture between foragers and farmers and/or changes in population structure during the period

between 5000 and 3500 BC in shaping the modern European gene pool.

Generally, little is known about the nature of the interactions between farmers and foragers during the Neolithic. It has been hypothesized to involve both hostile (13) and mutualistic interactions (14). There is also no real agreement on the intensity of contact; some authors claim limited or no contact, because early farmers occupied land that appears to have become devoid of Mesolithic people before their arrival (15, 16), whereas others suggest frequent contact and cooperation (17, 18).

Both hunter-gatherers and farmers used the Blätterhöhle as a burial place, and this indicates that they lived in geographically close, but distinct, niches. There may have been cultural contact between the two groups, an argument supported by the observation that—at least during the early Neolithic—flint technology traditions have a strong Mesolithic component (17, 19). The Blätterhöhle evidence is therefore consistent with a cultural contact model, whereby groups share the same or neighboring habitats, including burial places, but, nonetheless, adhere to their ancestral lifestyles and diet.

There are numerous ethnographic studies on modern hunter-gatherer communities living side

Table 1. Results of genetic and isotope analysis, and of ^{14}C dating in chronological order. Mesolithic samples are in bold; haplotype assignments from whole mtDNA genomes are marked with asterisk; samples falling into isotope-group 3 are highlighted in gray. Note that some samples, particularly in group 3, may have radiocarbon dates older than their true age because of the reservoir effect from freshwater fish consumption (4) (details on ^{14}C data are given in table S1 and fig. S1). Cells with hyphens represent no data available.

Lab code	^{14}C cal BC	mt hg	Isotopes			Isotope group
			$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{34}\text{S}$ (‰)	
BLA29	3020 ± 61	No U	−20.5	10.3	6.9	2
BLA28	3196 ± 103	J	−20.6	10.1	6.9	2
BLA26	3227 ± 90	-	−20.1	10.1	5.8	2
BLA5	3335 ± 136	H5	−19.8	10.3	5.9	2
BLA10*	3418 ± 63	H1c3*	−20.3	10.2	2.1	2
BLA25	3421 ± 63	U5b2a5	−19.9	10.3	6.3	2
BLA16	3429 ± 60	H11a	−20.2	10.1	4.2	2
BLA12	3449 ± 52	U5b2a2	−19.0	12.3	9.5	3
BLA1	3508 ± 102	U5b	−18.2	13.0	9.4	3
BLA13*	3513 ± 102	H5*	−20.1	10.4	5.3	2
BLA15	3571 ± 47	U5	−18.2	12.3	9.6	3
BLA21	3577 ± 43	U5	−19.0	12.2	9.3	3
BLA14*	3603 ± 49	U5b2b2*	−18.9	12.4	9.4	3
BLA24	3616 ± 56	U5	−18.7	12.5	9.5	3
BLA7*	3666 ± 20	H5*	−20.3	9.9	−0.1	2
BLA17	3681 ± 19	U5b2a2	−19.8	9.9	2.8	2
BLA9	3681 ± 19	U5b	−18.3	12.5	9.5	3
BLA8	3726 ± 38	U5	−18.8	12.3	8.7	3
BLA27	3869 ± 59	U5b2a2	−20.2	10.2	3.8	2
BLA11*	3922 ± 60	U5b2b(2)*	−18.4	12.0	9.6	3
BLA22	-	-	−19.8	10.5	4.5	2
BLA23	-	H	−20.7	10.3	5.3	2
BLA20*	8652 ± 58	U5a2c3*	−19.2	8.2	3.9	1
BLA19	8638 ± 56	U5a	−19.0	8.0	1.6	1
BLA2	8748 ± 67	U/K	−20.0	7.6	−1.8	1
BLA6	8796 ± 90	U5b2a2	-	-	-	-
BLA3	9210 ± 29	U2e	-	-	-	-

by side with farmers or herders (20, 21). In most cases, intergroup contact is common, e.g., the exchange of goods and food to complement their respective needs. Very often the exchange system follows a general “carbohydrates-for-protein” model, as various examples from the Philippines and Africa demonstrate [e.g. (22, 23)].

Despite these interactions, there are usually cultural norms regulating or restricting marriage between groups. Even though hunter-gatherer women can, under certain circumstances, assimilate into farmer or herder communities, this happens only rarely with hunter-gatherer men. Farming women tend not to join forager groups and mostly consider this as a social demotion (24, 25). Although our mitochondrial data only reflect the matrilineal history, these findings are consistent with the Blätterhöhle, where there is no evidence of introgression of farming females into the hunter-gatherer group; nevertheless, lineages formerly found in hunter-gatherers are also present in the group with a farming diet.

Conversely, in Northern Europe, the presence of lineages typical of farmers in the presumably

late Neolithic forager site of Ostorf (2) and in Scandinavian Pitted Ware people (26) could be interpreted as a sign of admixture in the opposite direction, i.e., women moving from farming communities to those with a hunter-gatherer lifestyle.

Undoubtedly, intermarriage between hunter-gatherers and farmers is likely to have been complex and variable across regions. Nonetheless, it is remarkable to find that a hunter-gatherer group and a group of Neolithic farmers led a parallel existence with a well-defined cultural boundary for more than 2000 years after the onset of the Neolithic in Central Europe.

It remains unclear whether the “parallel society” model we propose applies to other regions in Neolithic Europe, but the Blätterhöhle data provide the strongest evidence to date that genetically distinct hunter-gatherer groups survived for a much longer time than was previously assumed. Some of these late hunter-gatherers may have eventually converted to farming, the economy and lifestyle that became dominant for the following 5000 years.

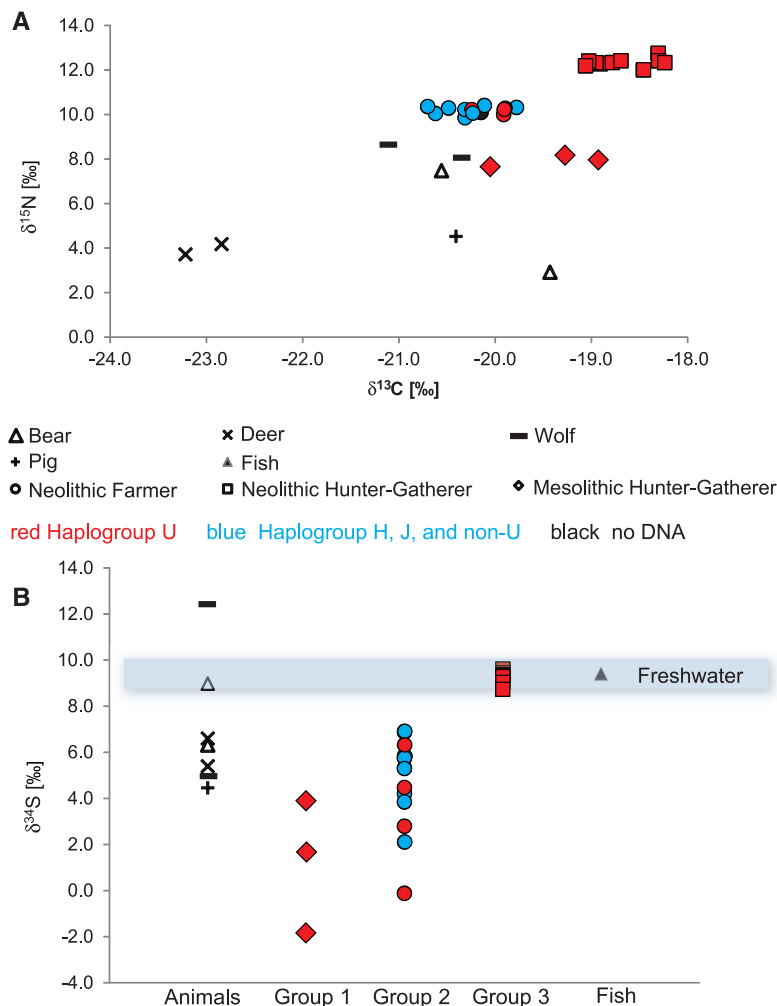


Fig. 2. Carbon, nitrogen, and sulfur isotope data of animals and humans from the Blätterhöhle. (A) Carbon and nitrogen. (B) Sulfur. Samples can be divided in three clusters: group 1 (diamonds) consists of Mesolithic foragers, group 2 (circles) comprises Neolithic farmers, and group 3 (squares) represents Neolithic fisher-hunter-gatherers.

References and Notes

- R. Pinhasi, M. G. Thomas, M. Hofreiter, M. Currat, J. Burger, *Trends Genet.* **28**, 496–505 (2012).
- B. Bramanti *et al.*, *Science* **326**, 137–140 (2009).
- J. Orschiedt, B. Gehlen, W. Schön, F. Gröning, *Notae Prehist.* **32**, 73–88 (2012).
- Supplementary materials are available on Science online.
- Q. Fu *et al.*, *Curr. Biol.* **23**, 553–559 (2013).
- M. P. Richards, R. E. M. Hedges, R. Jacobi, A. Current, C. Stringer, *J. Archaeol. Sci.* **27**, 1–3 (2000).
- E. Lightfoot, B. Boneva, P. T. Miracle, M. Slaus, T. C. O'Connell, *Antiquity* **85**, 73–86 (2011).
- A. Bogaard, T. H. E. Heaton, P. Poulton, I. Merbach, *J. Archaeol. Sci.* **34**, 335–343 (2007).
- O. Nehlich *et al.*, *J. Archaeol. Sci.* **36**, 1791–1799 (2009).
- V. M. Oelze *et al.*, *J. Archaeol. Sci.* **38**, 270–279 (2011).
- C. Bonsall *et al.*, *J. Eur. Archaeol.* **5**, 50–92 (1997).
- O. Nehlich, D. Boric, S. Stefanovic, M. P. Richards, *J. Archaeol. Sci.* **37**, 1131–1139 (2010).
- L. H. Keeley, in *Transitions to Agriculture in Prehistory*, A. B. Gebauer, T. D. Price, Eds. (Prehistory Press, Madison, WI, 1992), pp. 81–95.
- S. A. Gregg, *Forager and Farmers: Population Interaction and Agricultural Expansion in Prehistoric Europe* (Univ. of Chicago Press, Chicago, 1988).
- B. Vanmontfort, *J. Anthropol. Archaeol.* **27**, 149–160 (2008).
- S. Shennan, *Hum. Biol.* **81**, 339–355 (2009).
- D. Gronenborn, *J. World Prehist.* **13**, 123–210 (1999).
- A. Whittle, *Europe in the Neolithic: The Creation of New Worlds* (Cambridge World Archaeology, Cambridge Univ. Press, Cambridge, 1996).
- I. Mateiciucová, *Talking Stones: The Chipped Stone Industry in Lower Austria and Moravia and the Beginnings of the Neolithic in Central Europe (LBK, 5700–4900 BC)* (Masarykova Univ., Brno, Czech Republic, 2008).
- J. T. Peterson, *Am. Anthropol.* **80**, 335–351 (1978).
- T. N. Headland, L. A. Reid, in *Between Bands And States*, S. A. Gregg, Ed. (Centre for Archaeological Investigations, occas. pap. 9, Southern Illinois Univ., Carbondale, IL, 1991), pp. 333–340.
- J. F. Eder, in *Ethnic Diversity and the Control of Natural Resources in Southeast Asia*, T. Rambo, K. Gillogy, K. Hutterer, Eds. (Center for South and Southeast Asian Studies, University of Michigan, Ann Arbor, 1988), pp. 37–57.
- C. Turnbull, *Wayward Servants: The Two Worlds of the African Pygmies* (Natural History Press, Garden City, NY, 1965).
- P. Verdu *et al.*, *Mol. Biol. Evol.* **30**, 918–937 (2013).
- R. A. Bentley, R. H. Layton, J. Tehrani, *Hum. Biol.* **81**, 159–179 (2009).
- H. Malmström *et al.*, *Curr. Biol.* **19**, 1758–1762 (2009).
- L. Fiedler, *Führer zur hessischen Vor- und Frühgeschichte 2, Altsteinzeitliche Fundplätze in Hessen* (Theiss, Stuttgart, 1994).

Acknowledgments: We thank S. Shennan for discussion and comments on the manuscript and R. Blank, M. Baales, B. Gehlen, W. Schön, and A. Zimmermann for information and discussion. M. Unterländer and A. Schulz helped to generate the data. This study has been partly financed by the German Research Foundation (DFG: Or 98/6-1 and NE1666/1-1), the BEAN project of the Marie Curie Initial Training Networks Theme (grant no. 289966), the Max Planck Society and the Social Sciences and Humanities Research Council of Canada, the Synthesis Project of the European Union Seventh Framework Programme (grant no. 226506), and funds of the University of Mainz to J.B. Genetic sequences are deposited in GenBank (www.ncbi.nlm.nih.gov/), accession numbers KF523384 to KF523407.

Supplementary Material

www.sciencemag.org/content/342/6157/479/suppl/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S6
Tables S1 to S9
References (28–83)

22 August 2013; accepted 26 September 2013
Published online 10 October 2013;
10.1126/science.1245049

Changing Social Norm Compliance with Noninvasive Brain Stimulation

C. C. Ruff,^{1*} G. Ugazio,^{1,2} E. Fehr^{1*}

All known human societies have maintained social order by enforcing compliance with social norms. The biological mechanisms underlying norm compliance are, however, hardly understood. We show that the right lateral prefrontal cortex (rLPFC) is involved in both voluntary and sanction-induced norm compliance. Both types of compliance could be changed by varying the neural excitability of this brain region with transcranial direct current stimulation, but they were affected in opposite ways, suggesting that the stimulated region plays a fundamentally different role in voluntary and sanction-based compliance. Brain stimulation had a particularly strong effect on compliance in the context of socially constituted sanctions, whereas it left beliefs about what the norm prescribes and about subjectively expected sanctions unaffected. Our findings suggest that rLPFC activity is a key biological prerequisite for an evolutionarily and socially important aspect of human behavior.

Human societies depend crucially on social norms that specify the range of permissible actions for a given situation. Social norms range from the mundane (such as dress codes and table etiquette) to the profound (such as collective action, bilateral exchange, and obedience to the law). They are considered a hallmark of human civilization because no other known species regulates social interactions to the same degrees by norms (1–3). The potential of norms to guide collective behavior can break down if norm violations are not sanctioned, because humans tend to follow prevailing norms conditional on observing others' compliance (4). All known human societies have therefore enforced norm compliance by threatening norm violators with punishment, both officially via legal codes and institutions and informally in the context of private sanctions through peers (5, 6). The importance of credible sanctioning threats for maintaining norm compliance is well established by ethnographic evidence (1, 2), evolutionary theory (1, 3), and laboratory experiments (5, 6).

It has been proposed that the human brain may have developed neural processes that support norm enforcement by generating appropriate behavioral responses to social punishment threats (7–10). However, neuroscience studies on social norms have mostly focused on the neural basis of punishing others (11–14), whereas evidence for neural circuitry underlying sanction-induced compliance with norms is scarce. In mature adults, a brain network involving an area in the right lateral prefrontal cortex (rLPFC) is activated during non-compliant behavior triggered by social punishment threats (10). However, it is not possible to conclude from correlative functional magnetic resonance imaging (fMRI) findings that norm

compliance depends causally on neural activity in the rLPFC (15). Establishing such a causal dependence is crucial for our understanding of how social norm compliance develops in the context of brain maturation (16) and how it is pathologically altered and therapeutically amenable in the context of brain disorders (9).

We used transcranial direct current stimulation (tDCS) (17) to examine whether social norm compliance depends causally on neural processing in the previously identified rLPFC region (10). Participants engaged via computer terminals in anonymous social interactions that had real financial consequences. In every round, participants ("player A") received an amount of money units (MUs) and decided how much of it to transfer to a randomly assigned anonymous opponent ("player B"). In baseline rounds, this transfer was implemented, whereas in punishment rounds, player B could respond to the transfer by reducing player A's MUs [Fig. 1, fig. S1, and supplementary materials (18)]. In Western cultures, a fairness norm (19–21) pre-

scribes to split the "cake" of MUs equally between both players. This conflicts with player A's self-interest motive to keep as many MUs as possible. In baseline rounds, player A thus typically transfers only around 10 to 25% of the MUs. In contrast, when a sanctioning threat is present, player A largely obeys the fairness norm and transfers around 40 to 50% of the MUs (10, 20). The transfer difference between punishment and baseline rounds thus indexes sanction-induced norm compliance; that is, the degree to which the sanction threat induces player A to change her transfer from the level of voluntary norm compliance as measured in baseline rounds.

Individual differences in sanction-induced norm compliance correlate with fMRI-measured activity in the rLPFC (10). Based on this finding and the rLPFC's general role in the control of behavior (22, 23), it has been proposed that the rLPFC may weigh fair versus selfish responses specifically when punishment threats are present (8, 10). To provide causal evidence for this hypothesis, we first identified the specific rLPFC region described in (10), using MR scans of 63 female participants; we then experimentally altered neural excitability in this brain area during behavioral performance in a double-blind, placebo-controlled tDCS design (supplementary materials and fig. S2). tDCS can increase or decrease neural excitability in the stimulated region, depending on the polarity of the current flow (17). We thus randomly sorted participants into three stimulation groups, in which neural excitability in the rLPFC was enhanced with anodal tDCS, reduced with cathodal tDCS, or left unaltered by sham/placebo tDCS as control for possible non-neural effects of stimulation (supplementary materials). Such non-neural effects did not differ between the groups (supplementary materials) and therefore could not account for performance in the norm-compliance paradigm.

Participants were sensitive to the punishment threat and transferred more money in punishment

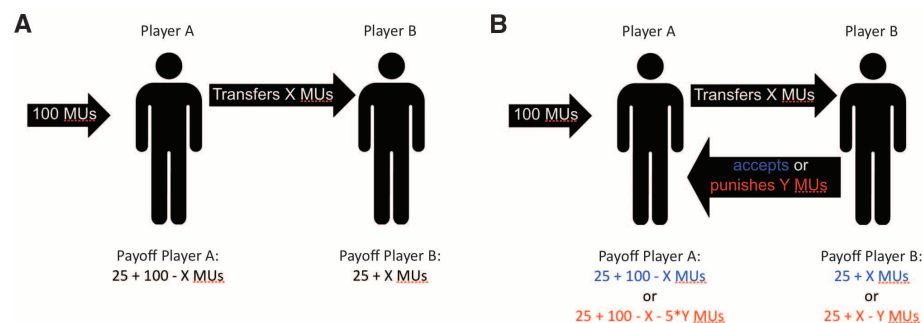


Fig. 1. Economic game used to measure social norm compliance. In each round, both players receive 25 MUs. Player A is given an additional 100 MUs that she can share with player B by sending a transfer X (in multiples of 10 MUs). All experimental MUs are exchanged for real money at the end of the experiment. Two types of rounds are presented in random order. **(A)** Baseline round: Transfer X is implemented as proposed, measuring player A's voluntary norm compliance. **(B)** Punishment round: Player B can either accept X (blue font) or invest Y MUs from her initial endowment to punish player A (red font). Y can be any integer between 0 and 25, reducing A's payoff by $5 \times Y$ MUs. Player A is aware of this possible sanction; any increase in transfers for punishment relative to baseline rounds therefore measures sanction-induced norm compliance.

¹Laboratory for Social and Neural Systems Research (SNS-Lab), Department of Economics, University of Zurich, Zurich, Switzerland. ²Social, Cognitive and Affective Neuroscience Unit, University of Vienna, Vienna, Austria.

*Corresponding author. E-mail: christian.ruff@econ.uzh.ch (C.C.R.); ernst.fehr@econ.uzh.ch (E.F.)

than in baseline rounds [mean transfer difference, 29.44 MUs; $P < 0.001$, generalized least squares (GLS) regression]. However, in line with our hypothesis, the two active brain stimulation conditions changed sanction-induced norm compliance in opposite ways relative to the sham condition (Fig. 2A and table S2). Anodal tDCS increased the transfer difference by 33.5% (GLS regression, $P < 0.001$), whereas cathodal tDCS decreased the transfer difference by 22.7% ($P < 0.001$).

Do these effects reflect changes in altruistic behavior, with increased or decreased monetary transfers regardless of punishment threats? This interpretation is refuted by the data on voluntary norm compliance in baseline rounds (Fig. 2B and table S3). Voluntary transfers were actually decreased (GLS regression, $P < 0.001$) during anodal tDCS and increased ($P < 0.01$) during cathodal tDCS, relative to the sham condition. This not only confirms that tDCS affected participants' response to the punishment threat but that these tDCS effects on sanction-induced compliance

were actually stronger than the opposite effects on voluntary compliance: If tDCS had not affected sanction-induced compliance, then overall transfers in punishment rounds, which are based on voluntary plus sanction-induced compliance, should also be lower after anodal and higher after cathodal stimulation. However, overall transfers in punishment rounds were in fact higher (GLS regression, $P < 0.05$) during anodal tDCS and lower ($P < 0.001$) during cathodal tDCS than in the sham condition (fig. S3).

Which task-related psychological mechanisms may have contributed to the tDCS effects? To respond appropriately, participants need to know the fairness norm and form appropriate beliefs about player B's reactions. We measured (i) the participants' perceived fairness, (ii) the anger they expected the opponent to feel, and (iii) the punishment they expected at different transfer levels (Fig. 3). All participants were clearly aware of the fairness norm and rated higher transfers as significantly fairer [analysis of var-

iance, $F(2,60) = 84.88$, $P < 0.001$], less likely to cause anger in the opponent [$F(2,60) = 218.96$, $P < 0.001$], and leading to lower punishment [$F(2,60) = 82.69$, $P < 0.001$]. Importantly, the type of brain stimulation did not affect participants' beliefs, neither on average [all $F(2,60) < 0.94$, all $P > 0.39$] nor in their change across different transfer levels [all $F(2,60) < 0.55$, all $P > 0.74$].

Our findings do not yet show that the stimulated rLPFC region implements specifically social aspects of behavioral control. In particular, behavior in punishment rounds requires risk-taking and trading off higher transfers with a lower risk of sanction. We therefore repeated the experiment in a sample of 59 new female volunteers who took the identical decisions as before, but now played against a computer pre-programmed to respond in the same way as a human opponent in punishment rounds (supplementary materials). In this "nonsocial context," participants were also sensitive to punishment threats (fig. S4A), but the effects of tDCS on sanction-induced transfers were significantly weaker than during interactions with human opponents (Fig. 4A and table S3). This held for both increases in sanction-induced transfers due to anodal tDCS (GLS regression, $P = 0.009$) and decreases due to cathodal tDCS ($P = 0.001$, GLS regression). In baseline rounds of the nonsocial context, where no social norm prescribes sharing MUs with the computer, participants hardly transferred any MUs (fig. S4B). Such (possibly erroneous) voluntary transfers to the computer were therefore also less affected by tDCS than norm-related voluntary transfers to human opponents (Fig. 4B; GLS regression, $P < 0.05$ for anodal tDCS and $P < 0.001$ for cathodal tDCS).

Social punishment is thought to have played an important role in the evolution of human social behavior and cooperation (1–3). Our results show that the influence of punishment threats on human social norm compliance depends causally on neural activity in the rLPFC. This suggests a neural mechanism involving the rLPFC that aligns behavior with social norms when punishment is possible. The more pronounced involvement of this mechanism for genuinely social punishments concurs with suggestions that during human brain evolution, the steep increase in the complexity of social interactions may have shaped specific neural processes for social behavior (8, 24). That tDCS affected sanction-induced and voluntary norm compliance in opposite ways suggests that these two forms of norm compliance involve distinct neural circuits; in particular, the rLPFC seems to play a fundamentally different role in voluntary and sanction-based norm compliance.

Our finding that rLPFC stimulation did not affect awareness of the fairness norm and expected sanctions suggests that the rLPFC process necessary for norm-compliant behavior is dissociated from neural mechanisms enabling humans to anticipate sanctions for norm violations and to distinguish "right" from "wrong." The rLPFC

Fig. 2. rLPFC stimulation changes sanction-induced and voluntary norm compliance. (A) Sanction-induced norm compliance: Average ($\pm$ SEM) transfer difference for punishment rounds minus baseline rounds. Higher values indicate that the punishment threat led to a larger adjustment of transfers toward the fairness norm of an equal split. (B) Voluntary norm compliance: Average ($\pm$ SEM) transfers for baseline rounds. All values were determined with the regression in eq. S1; * $P < 0.05$.

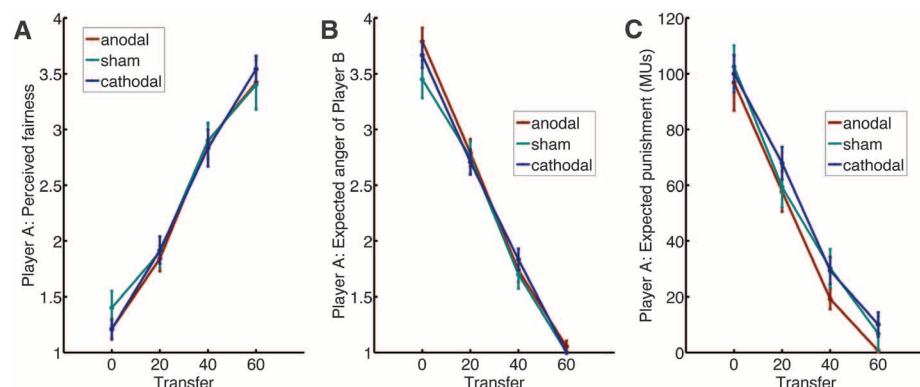
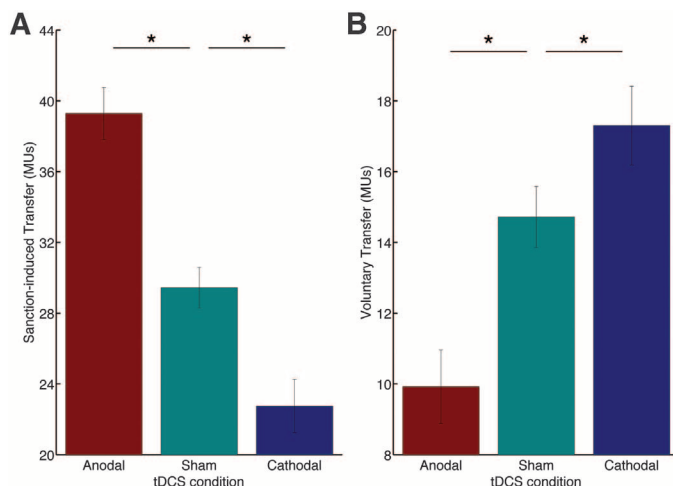


Fig. 3. rLPFC stimulation does not affect participants' beliefs about the fairness of different transfers and about player B's anticipated anger and expected punishment. (A) Average rating of perceived fairness for different transfer levels [scale varies from 1 ("very unfair") to 4 ("very fair")]. (B) Average rating of anticipated anger felt by player B for different transfer levels [scale varies from 1 ("not angry at all") to 4 ("very angry")]. (C) Average expected payoff reduction resulting from B's punishment. Error bars represent SEM.

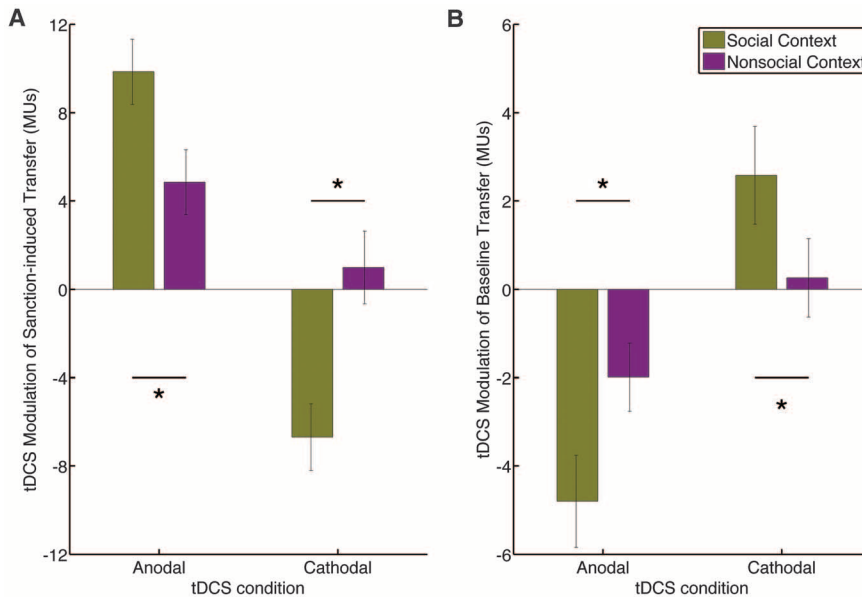


Fig. 4. rLPFC stimulation effects are stronger during social interactions. (A) tDCS effects on sanction-induced norm compliance during interactions with a human (Social Context) or a computer opponent (Nonsocial Context). Bars depict average changes in transfer difference for anodal and cathodal tDCS relative to the sham condition. (B) tDCS-related changes of voluntary transfers in baseline rounds. Bars represent average changes for anodal and cathodal tDCS relative to the sham condition. All values were determined with regression in eq. S2; * $P < 0.05$.

mechanism necessary for norm compliance is probably not restricted to neural activity within this brain area, given that the prefrontal cortex is involved in many aspects of behavioral control (23) and that brain stimulation can affect areas interconnected with the stimulation site (25). The anatomical connectivity (26) and context-dependent functions of the prefrontal cortex (27) make it more likely that the stimulated rLPFC area integrates and coordinates activity in a network of brain regions triggered by the need for considering social punishments during action control (8).

Brain stimulation studies in humans have so far mostly shown unidirectional maladaptive effects on decision-making, rendering participants more impulsive (28), selfish (29), or cognitively biased (30). Such interventions may therefore be of limited practical use in applied settings. Our finding that changes in the neural excitability of the rLPFC can enhance voluntary and sanction-induced social norm compliance may be of relevance because noncompliance with social norms constitutes a major problem in neurological (31) and psychiatric (31, 32) disorders, during abnormal development in adolescence (33), and in adults in the form of criminal activity (9). However, the opposite influence of brain stimulation on

voluntary and sanction-induced norm compliance also suggests that increasing one type of norm compliance with brain stimulation may come at the cost of decreasing the other type.

References and Notes

1. E. Sober, D. S. Wilson, *Unto Others—The Evolution and Psychology of Unselfish Behavior* (Harvard Univ. Press, Cambridge, MA, 1998).
2. S. Mathew, R. Boyd, *Proc. Natl. Acad. Sci. U.S.A.* **108**, 11375–11380 (2011).
3. R. Boyd, H. Gintis, S. Bowles, P. J. Richerson, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 3531–3535 (2003).
4. U. Fischbacher, S. Gächter, E. Fehr, *Econ. Lett.* **71**, 397–404 (2001).
5. R. Forsythe, H. L. Horowitz, N. E. Savin, M. Sefton, *Games Econ. Behav.* **6**, 347–369 (1994).
6. E. Fehr, S. Gächter, *Nature* **415**, 137–140 (2002).
7. P. R. Montague, T. Lohrenz, *Neuron* **56**, 14–18 (2007).
8. J. W. Buckholz, R. Marois, *Nat. Neurosci.* **15**, 655–661 (2012).
9. A. Raine, Y. Yang, *Soc. Cogn. Affect. Neurosci.* **1**, 203–213 (2006).
10. M. Spitzer, U. Fischbacher, B. Herrnberger, G. Grön, E. Fehr, *Neuron* **56**, 185–196 (2007).
11. A. G. Sanfey, J. K. Rilling, J. A. Aronson, L. E. Nystrom, J. D. Cohen, *Science* **300**, 1755–1758 (2003).
12. D. J. de Quervain *et al.*, *Science* **305**, 1254–1258 (2004).
13. J. W. Buckholz *et al.*, *Neuron* **60**, 930–940 (2008).
14. M. J. Crockett *et al.*, *J. Neurosci.* **33**, 3505–3513 (2013).

15. For example, individuals with particular personality traits, such as anxious individuals or those with a tendency for Machiavellian behavior, may have generally higher LPFC activity and a generally higher propensity to respond to sanctions, but apart from their covariation, these two variables may not directly influence one another. Alternatively, it is also possible that rather than being the cause of norm compliance, brain activation in the rLPFC is merely the consequence of norm compliance. In other words, individuals who respond more strongly to the sanctioning threat may recruit the rLPFC more strongly as a result of their choice.
16. N. Steinbeis, B. C. Bernhardt, T. Singer, *Neuron* **73**, 1040–1051 (2012).
17. M. A. Nitsche, W. Paulus, *J. Physiol.* **527**, 633–639 (2000).
18. Methods and materials, supplementary analyses, and supplementary figures are available as supporting material on Science Online.
19. E. Fehr, U. Fischbacher, *Evol. Hum. Behav.* **25**, 63–87 (2004).
20. C. F. Camerer, *Behavioral Game Theory: Experiments in Strategic Interaction* (Princeton Univ. Press, Princeton, NJ, 2003).
21. J. Henrich *et al.*, *Am. Econ. Rev.* **91**, 73–78 (2001).
22. A. R. Aron, T. W. Robbins, R. A. Poldrack, *Trends Cogn. Sci.* **8**, 170–177 (2004).
23. E. K. Miller, J. D. Cohen, *Annu. Rev. Neurosci.* **24**, 167–202 (2001).
24. R. I. M. Dunbar, *Trends Cogn. Sci.* **16**, 101–102 (2012).
25. J. Driver, F. Blankenburg, S. Bestmann, W. Vanduffel, C. C. Ruff, *Trends Cogn. Sci.* **13**, 319–327 (2009).
26. P. L. Croxson *et al.*, *J. Neurosci.* **25**, 8854–8866 (2005).
27. J. Duncan, *Trends Cogn. Sci.* **14**, 172–179 (2010).
28. B. Figner *et al.*, *Nat. Neurosci.* **13**, 538–539 (2010).
29. D. Knoch, A. Pascual-Leone, K. Meyer, V. Treyer, E. Fehr, *Science* **314**, 829–832 (2006).
30. G. Xue, C. H. Juan, C. F. Chang, Z. L. Lu, Q. Dong, *Proc. Natl. Acad. Sci. U.S.A.* **109**, 4401–4406 (2012).
31. A. Bechara, M. Van Der Linden, *Curr. Opin. Neurol.* **18**, 734–739 (2005).
32. B. King-Casas *et al.*, *Science* **321**, 806–810 (2008).
33. R. Loeber, D. P. Farrington, *Dev. Psychopathol.* **12**, 737–762 (2000).

Acknowledgments: C.C.R. and E.F. designed the research, C.C.R. and G.U. conducted the research, C.C.R. and G.U. analyzed data with input from E.F., and C.C.R. and E.F. wrote the paper with comments by G.U. We thank K. Treiber and A. Schlaepfer for their assistance with data collection. This study was supported by a European Research Council grant to E.F. (Foundations of Economic Preferences), Swiss National Science Foundation (SNSF) grants to C.C.R. and E.F. (CRSII3_141965 and 51NF40_144609), and the SNSF-funded Swiss National Center of Competence in Research Affective Sciences.

Supplementary Materials

www.sciencemag.org/content/342/6157/482/suppl/DC1
Materials and Methods
Figs. S1 to S4
Tables S1 to S4
References (34, 35)

3 June 2013; accepted 24 September 2013
Published online 3 October 2013;
10.1126/science.1241399

WATER SORPTION ANALYZER

The Aquadyne DVS-2HT is the latest in the family of precision water sorption analyzers, joining the Aquadyne DVS-1, single-balance unit, and the Aquadyne DVS-2 dual-balance unit. The Aquadyne DVS water sorption analyzer is capable of analysis temperatures from 10°C up to 85°C. The Aquadyne DVS instruments are used to accurately measure the amount of water vapor a sample can adsorb, and the rate at which it is adsorbed and desorbed. This is accomplished by gravimetrically monitoring the process while precisely controlling the amount of water in a flowing stream of nonreactive gas; a technique referred to as dynamic vapor sorption (DVS). The instruments use precision microbalances housed in a temperature-controlled housing, which enables the measurement of miniscule changes in weight in the microgram range. This high sensitivity along with precise temperature and humidity control ensures accurate, reproducible results every time.

Quantachrome Instruments

For info: 800-989-2476 | www.quantachrome.com

**BASEMENT MEMBRANE EXTRACTS**

Two new formulations of Basement Membrane Extract (BME) are now available: Cultrex BME 2 (organoid growth matrix) and Cultrex BME 3 (xenograft/tumor graft matrix). The new products have been developed to address the heterogeneous nature of tissue and organ microenvironments that not only arise from organ specific stromal cells, growth factors, proteoglycans, and protein composition but also from the stiffness or tensile strength of the BME. BME 2 is based upon a proprietary formulation that is higher in tensile strength than original Matrigel or Cultrex BME. This growth factor reduced product is recommended for organoid growth applications including 3-D cell culture from stem cells. By comparison, BME 3 has been developed to be physiologically aligned with the in vivo tumor environment and is recommended for xenografts, tumor grafts, and other in vivo applications. Cultrex BME is a soluble form of basement membrane purified from Engelbreth-Holm-Swarm tumors.

AMS Biotechnology

For info: +44-(0)-1235-828200 | www.amsbio.com

METABOLITE-TESTING REAGENT

New metabolite-testing reagent sets have been designed to determine the concentration of iron and phosphate, which are two parameters relevant for cell growth and productivity during fermentation processes. The tests are designed for applications in research and bio-manufacturing process development and will allow the measurement of iron (complexed iron/ Fe^{3+} ions) and phosphate on Cedex Bio instruments. The new tests can be easily installed by barcode reading through the customer supporting the flexible testing concept. Offered as liquid-stable formulation, they are ready for use and do not require manual dilution or pipetting steps. With their increased precision, accuracy, and long calibration intervals, the new iron and phosphate tests offer cost-effective and convenient testing with less hands-on time for users.

Roche

Phone: +49-88-56601-0210 | www.roche.com

MOLECULAR ACTIVITY ANALYSIS

The ground breaking new science in Activity Miner helps researchers optimize their leads by identifying the key 3-D structural and electrostatic changes that impact molecular activity. Activity Miner explores the structure activity landscape of a set of molecules. For each pair of molecules, the difference between them in electrostatics, shape, and structure is compared to the difference in activity. A small change in structure or electrostatics that results in a large change in activity is known as an activity cliff. Conversely, large structural changes resulting in little or no change in activity indicate bioisosteres. Activity Miner uses Cresset's molecular fields so that 3-D electrostatic and shape similarities can be analyzed as well as 2-D structural activity. This makes it meaningful to compare pairs of structurally diverse compounds and gives a more realistic insight into activity relationships. Activity Miner is available for beta testing over the summer. The full version will be available in the next release of Cresset's Forge and Torch.

Cresset

For info: +44-(0)-1707-356120 | www.cresset-group.com

AUTOMATED TISSUE PROCESSOR

The unique state-of-the-art tissue processor, LYNX II, is not only compatible with all plastic resins but with paraffin waxes as well. LYNX II is the successor of Lynx Tissue Processor with several enhancements, including capabilities to perform optional processing of larger size samples for histology. The LYNX II holds 24 reagent vials for electron microscopy (EM) processing and provides an option for histology processing (HP) using 12 larger size reagent vials. In both EM and HP modes, LYNX II has two independently controlled heating/cooling stations. The tissue processor features 10 programs for EM and 10 programs for HP; two independently controlled heating/cooling stations; easy replacement of reagent carousel; paraffin processing; sample agitation; vacuum infiltration during processing; and a choice of plastic and metal containers for improved chemical compatibility and heat transfer. An optional external media-based interface to a PC is offered for ease of programming.

Electron Microscopy Sciences

For info: 215-412-8400 | www.emsdiasum.com

Electronically submit your new product description or product literature information! Go to www.sciencemag.org/products/newproducts.dtl for more information. Newly offered instrumentation, apparatus, and laboratory materials of interest to researchers in all disciplines in academic, industrial, and governmental organizations are featured in this space. Emphasis is given to purpose, chief characteristics, and availability of products and materials. Endorsement by Science or AAAS of any products or materials mentioned is not implied. Additional information may be obtained from the manufacturer or supplier.



Join Us in Chicago

Learn about the science and technology that is addressing current and future global challenges.

- Seminars on innovation, entrepreneurship, and the economy; big data; communicating science; and food security and sustainability.
- 150+ symposia in 16 disciplinary tracks covering the latest research advances.
- Connect with colleagues in all fields of science, technology, and engineering and attend career development workshops.

Browse full program and register:
www.aaas.org/meetings

Connect with us



@AAASmeetings #AAASmtg



www.facebook.com/AAAS.Science

Reporters: The EurekAlert! website hosts the AAAS Annual Meeting Newsroom. Reporters can obtain details and register at www.eurekalert.org/newsroom



ADVANCING SCIENCE, SERVING SOCIETY

AAAS presents the

2014 AAAS ANNUAL MEETING

MEETING GLOBAL CHALLENGES: DISCOVERY AND INNOVATION



Dear Colleagues,

On behalf of the AAAS Board of Directors, it is my honor to invite you to join us in Chicago for the 2014 AAAS Annual Meeting, 13-17 February. This annual event is one of the most widely recognized global science gatherings, with hundreds of diverse scientific sessions and communication opportunities with broad U.S. and international media coverage.

This year's theme—*Meeting Global Challenges: Discovery and Innovation*—will focus on finding sustainable solutions through inclusive, international, and interdisciplinary efforts that are most useful to society and enhance economic growth.

Scientific discovery and innovation are driving solutions to current and future global challenges, including sufficient food, quality healthcare, renewable fuels, and a sustainable environment. Addressing these challenges depends upon international dialogue and discoveries emerging from the convergence of physical, life, engineering, and social sciences in innovative ways that are most useful to society. The scientific program will highlight the increasing interdependence of economic progress and advances in science and technology.

Everyone is welcome at the AAAS Annual Meeting. Those who attend will have the opportunity to choose among a broad range of activities, including plenary and topical lectures by some of the world's leading scientists and engineers, multidisciplinary symposia, cutting-edge seminars, career development workshops, and an international exhibition. You and your family can also enjoy Family Science Days, a free event open to the public.

The Annual Meeting reflects tremendous efforts from the AAAS sections, divisions, and committees. I also acknowledge the members of the Scientific Program Committee who selected and assembled the many excellent ideas and proposals into this outstanding meeting.

We look forward to seeing you in Chicago.

Phillip A. Sharp
AAAS President and Program Chair
Institute Professor, Koch Institute for Integrative Cancer Research
Massachusetts Institute of Technology



President's Address

Thursday, 13 February



Phillip A. Sharp

Institute Professor, Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology

Dr. Sharp, a noted molecular biologist with a focus on the genetic causes of cancer, shared the 1993 Nobel Prize in Physiology or Medicine for his discovery of “split genes”—the finding that genes could be composed of several separate segments within DNA. His lab now focuses on the therapeutic potential of RNA interference, small RNA molecules that can switch genes on and off. He has co-founded two companies: Biogen (now Biogen Idec) and Alnylam Pharmaceuticals. He received a Ph.D. in chemistry from the University of Illinois at Urbana-Champaign and is an elected member of the National Academy of Sciences, the Institute of Medicine, AAAS, the American Academy of Arts and Sciences, the American Philosophical Society, and a foreign fellow of the Royal Society (U.K.).

Plenary Speakers



Friday, 14 February

Steven Chu

Professor of Physics and Molecular and Cellular Physiology, Stanford University

How Discovery and Innovation Can Meet Our Energy Challenge

Dr. Chu served as the 12th U.S. Secretary of Energy between January 2009 and April 2013. Prior to his post in President Obama's Cabinet, he was the director of Lawrence Berkeley National Laboratory

and a professor at University of California, Berkeley. He had previously worked at Stanford University and Bell Laboratories. Chu is the co-recipient of the Nobel Prize for Physics (1997) for his contributions to the laser cooling and trapping of atoms. His other areas of research include tests of fundamental theories in physics, atom interferometry, study of polymers and biological systems at the single molecule level, and biomedical research. The holder of 10 patents, Chu has published 250 scientific and technical papers. Chu is a member of the National Academy of Sciences, the American Philosophical Society, the Royal Academy of Engineering, the Academia Sinica, and the Korean Academy of Sciences and Technology, an honorary member of the Institute of Physics and the Chinese Academy of Sciences, and a Lifetime Member of the Optical Society of America. He received a bachelor's degree in physics from the University of Rochester and a Ph.D. in physics from the University of California, Berkeley.

Saturday, 15 February



Alan Alda

Visiting Professor of Journalism, Stony Brook University

Getting Beyond a Blind Date with Science

Alan Alda is an actor, writer, director, and visiting professor at the Alan Alda Center for Communicating Science at Stony Brook University, where he helps current and future scientists learn to communicate more clearly and vividly with the public. In collaboration with theater arts faculty at Stony Brook, he is pioneering the use of improvisational theater exercises to help scientists connect more directly with people outside their field. Alda is best

known for his award-winning work in movies, theater, and television, but he also has a distinguished record in the public communication of science. For 13 years he hosted the PBS series *Scientific American Frontiers*, which he has called “the best thing I ever did in front of a camera.” After interviewing hundreds of scientists around the world, he became convinced that many researchers have wonderful stories but need to learn how to tell them better. That realization inspired the creation of Stony Brook's multidisciplinary Alan Alda Center for Communicating Science in 2009.

Sunday, 16 February



Susan Lindquist

Professor of Biology, Massachusetts Institute of Technology

From Yeast Cells to Patient Neurons: A Powerful Discovery Platform for Parkinson's and Alzheimer's Diseases

Dr. Lindquist is a pioneer in understanding protein folding, showing that these changes can have profound and unexpected influences in human disease, evolution, and nanotechnology. She is a member of the Whitehead Institute, where she served as director from 2001 to 2004, and a Howard Hughes Medical Institute investigator. Previously she was a professor of medical sciences and molecular biology at University of Chicago. Lindquist is an elected fellow of the American Academy of Arts and Sciences, the National Academy of Sciences, the Institute of Medicine, and the American Philosophical Society. She is a recipient of the Novartis/Drew Award for Biomedical Research, the Dickson Prize in Medicine, the Sigma Xi William Procter Prize for Academic Achievement, the Nevada Silver Medal for Scientific Achievement, the Genetics Society of

CONTINUED >



America Medal, and the Centennial Medal of the Harvard University Graduate School of Arts and Sciences. In 2010, she received the Mendel Medal from the Genetics Society (U.K.), the Delbruck Medal from Bayer Schering, and the National Medal of Science. She is a member of the scientific advisory board of the Institute for Molecular Biotechnology in Austria. Lindquist is a co-founder of FoldRx Pharmaceuticals, Inc., a subsidiary of Pfizer, Inc.

Monday, 17 February



John A. Rogers

Swanlund Chair and Professor of Materials Science and Engineering, University of Illinois, Urbana-Champaign

Stretchy Electronics That Dissolve in Your Body

Dr. Rogers' research includes fundamental and applied aspects of nano- and molecular scale fabrication. He also studies materials and patterning techniques for unusual electronic and photonic devices, with an emphasis on bio-integrated and bio-inspired systems. He received a Ph.D. in physical chemistry from Massachusetts Institute of Technology in 2005. He has published more than 350 papers and is an inventor on over 80 patents and patent applications, many of which are licensed or in active use by large companies and startups that he co-founded. He previously worked for Bell Laboratories as director of its research program in condensed matter physics. He has received recognition including a MacArthur Fellowship from the John D. and Catherine T. MacArthur Foundation, the Lemelson-MIT Prize, the National Security Science and Engineering Faculty Fellowship from the U.S. Department of Defense, the George Smith Award from IEEE, the Robert Henry Thurston Award from American Society of Mechanical Engineers, the Mid-Career Researcher Award from Materials Research Society, the Leo Hendrick Baekeland Award from the American Chemical Society, and the Daniel Drucker Eminent Faculty Award from the University of Illinois.

Topical Lectures

Additional speakers to be announced.



Cori Bargmann

Torsten N. Wiesel Professor
 Rockefeller University
Using Fixed Circuits to Drive Variable Behaviors



Eli Finkel

Professor of Psychology and Management and Organizations
 Northwestern University
The Suffocation of Marriage



Heinrich Jaeger

William J. Friedman and Alicia Townsend Professor of Physics
 University of Chicago
Granular Matter: From Basic Questions to New Concepts and Applications



Edward Roberts

David Sarnoff Professor of Management of Technology
 Massachusetts Institute of Technology
Entrepreneurial Impact of Science and Technology-Based Universities



Diana Wall

University Distinguished Professor of Biology
 Colorado State University
Lessons from an Antarctic Desert: Documenting Climate Change and Measuring Impact on Soil Life

Special Sessions

International Public Science Events Conference

Wednesday, 12 February—Thursday, 13 February
Pre-registration required

Responsible Professional Practices in a Changing Research Environment Workshop

Thursday, 13 February
Pre-registration required



Seminars

Thursday, 13 February

Communicating Science

Scientific and technological issues increasingly trigger societal conflicts whenever they intersect with personal or political views. Today's scientists and engineers are challenged to communicate and engage with the public and journalists, particularly amid pressures on research and development budgets and related concerns about transparency and accountability. This seminar will share science communication expertise in working with different types of content, across a range of formats, for various audiences.

Engaging with Journalists

Collaborator: Kavli Foundation and AAAS Kavli Science Journalism Awards

Moderator: Cornelia Dean, *The New York Times* and Brown University, New York City

SPEAKERS

Robert Lee Hotz, *The Wall Street Journal*, New York City
Carl Zimmer, science writer, Guilford, CT
Sarah Holt, independent television producer, Newton, MA
David Baron, Public Radio International, Boulder, CO

Engaging with Social Media

Speakers to be announced.

Engaging with Public Events

Collaborator: Science Festival Alliance and the International Public Science Events Conference

Moderator: Ben Lillie, The Story Collider, New York City

SPEAKERS

Rabiah Mayas, Museum of Science and Industry, Chicago, IL
Never Too Young: Museum-Based Approaches to Connecting Youth with Scientists

Kishore Hari, Bay Area Science Festival, San Francisco, CA
The Science Education Melting Pot

Amy Rowat, University of California, Los Angeles
Engaging General Audiences in Science Through Interactive Events

Friday, 14 February

Innovation, Entrepreneurship, and the Economy

This seminar will consider opportunities for innovation and entrepreneurship to benefit the economy. Dynamic examples of innovation and challenges in advanced manufacturing and biomedical research will be considered, and strategies to encourage entrepreneurship activity will be explored.

New Business Models for Accelerating Biomedical Innovation

Organized by: Andrew W. Lo, Massachusetts Institute of Technology, Cambridge

SPEAKERS

John McKew, National Center for Advancing Translational Science (NCATS), Rockville, MD
NCATS: Catalyzing Innovation

Pablo Legorreta, Royalty Pharma, New York City
Drug Royalty Investment Companies as Catalysts for Innovation

Bruce Lehmann, University of California, San Diego

Some Simple Economics for Early Stage Drug Development

Science-Driven Entrepreneurship: Determined Pursuit of Innovative Success

Organized by: Anice Anderson, Private Engineering Consulting, Carmel, IN

SPEAKERS

John M. Newsam, Tioga Research, Inc., San Diego, CA
Launching a Science-Based Enterprise with an Organic Growth Model

Irwin Jacobs, Qualcomm, San Diego, CA
Qualcomm: From Startup to Leadership in Technology and the Social Impact of 6.6 Billion Wireless Connections

Han Cao, BioNano Genomics Inc., San Diego, CA
Commercializing Innovation: Applying Nanotechnology to Genomics

Organizing the Innovation System for Advanced Manufacturing

Organized by: Stephanie Shipp, Virginia Tech, Arlington; William B. Bonvillian, Massachusetts Institute of Technology, Cambridge

SPEAKERS

Hod Lipson, Cornell University, Ithaca, NY

The Future of 3D Printing: Promise and Peril of a Machine that Can Make (Almost) Anything

Rodney Brooks, Rethink Robotics, Boston, MA
Robotics as a Transformative Manufacturing Technology: Status and Future

Jonas Nahm, Massachusetts Institute of Technology, Cambridge
The Manufacturing Economies in China and Germany: Technology and Process Systems

Suzanne Berger, Massachusetts Institute of Technology, Cambridge
Defining the Innovation Ecosystem for Advanced Manufacturing

Theresa Kotanchek, Evolved Analytics Inc, Midland, MI
Implementing the Advanced Manufacturing Partnership: Progress and Remaining Gaps

Jason Miller, White House National Economic Council, Washington, DC
National Manufacturing Institutes: What Are the Innovation Design Lessons?

Saturday, 15 February

Big Data: Applications and Implications

Innovations in big data are providing challenging new ways to understand large datasets with a wide range of potential applications from biology and medicine to research on urban environments. Realizing the benefits of big data will require an understanding of scientific, legal, policy, and societal implications.

How Big Data Supports Biomedical Discovery

Organized by: Robert L. Grossman, University of Chicago, IL

SPEAKERS

Brian D. Athey, University of Michigan, Ann Arbor
The transSMART Open Data Sharing and Analytics Cloud Platform

Lincoln Stein, Ontario Institute for Cancer Research, Toronto, Canada
The Cancer Genome Collaboratory

Robert L. Grossman, University of Chicago, IL
Supporting a Biomedical Commons with the Bionimbus Protected Data Cloud

Data Availability: Making Sure the Gift Keeps Giving

Organized by: Clifford Spiegelman, Texas A&M University, College Station

SPEAKERS

David Reitze, California Institute of Technology, Pasadena

Big Science, Big Data, Big Challenges: Data from Large-Scale Physics Experiments

Matt Ehling, Public Record Media, St. Paul, MN
Access to Government Data: Examining and Overcoming Barriers

Catherine Grosso, Michigan State University, East Lansing

Finding Data: The Politics and Magic of Accessing Capital Punishment Data

A New Era for Urban Research: Open Data and Big Computation

Organized by: Charlie Catlett, Argonne National Laboratory, IL

SPEAKERS

Philip Enquist, Skidmore, Owings and Merrill, Chicago, IL

Cities, Livability, and Responsibility to the Planet

Steven E. Koonin, New York University Center for Urban Science and Progress, Brooklyn
The Promise of Urban Science

Karen Weigert, City of Chicago, IL
Science-Driven Sustainability Policies in Chicago

Andrew Yao, Tsinghua University, Beijing, China
Urban Sensing and Informatics

Robert Sampson, Harvard University, Cambridge, MA
Ecometrics in the Age of Big Data: Measuring Urban Social Processes and Inequality

Mario Small, University of Chicago, IL
Poverty and Organizational Density

Sunday, 16 February

Food Security and Sustainability

Transformative solutions for sustainable food production are needed as global population approaches 9 billion by 2050 and climate change alters environmental landscapes and resources. The importance of undertaking agricultural research that enables governments to meet food demands and reduce shortages while developing environmentally sound and sustainable food production systems will be discussed. Recent advances in perennial grain crop development, from genomic innovations to real-world results on farms, will be conveyed.

Feeding a Growing Population While Sustaining the Earth

Organized by: Felix Kogan and Alfred M. Powell, National Oceanic and Atmospheric Administration (NOAA), College Park, MD

SPEAKERS

Thomas R. Karl, NOAA, Asheville, NC
Precipitation Changes in a Warmer World for Major Grain Growing Regions

Paul R. Ehrlich, Stanford University, CA
Feeding 9 Billion and Avoiding a Collapse of Civilization: Science's Main Challenge

Jonathan A. Foley, University of Minnesota, St. Paul
Challenges to Global Food Security and Environmental Sustainability

Felix Kogan, NOAA, College Park, MD
Overexploitation of Earth Resources, Climate Constraints and Food Security

Research and Development for Sustainable Agriculture and Food Security

Organized by: Daniel Bush, Colorado State University, Fort Collins

SPEAKERS

Jerry Hatfield, U.S. Department of Agriculture (USDA), Ames, IA
Natural Resources: The Overlooked Component in Food Security and Sustainable Agriculture

Philip Pardey, University of Minnesota, St. Paul
The Changing Global Landscape for Food and Agricultural Research and Development

Wendy Wintersteen, Iowa State University, Ames
Public-Private Partnerships to Achieve Food Security and Sustainable Agriculture

Perennial Grains for Food Security in a Changing World: Gene to Farm Innovations

Organized by: Jerry Glover, U.S. Agency for International Development (USAID), Washington, DC; Sieglinde S. Snapp, Michigan State University, Hickory Corners

SPEAKERS

Wezi Mhango, Lilongwe University of Agriculture and Natural Resources, Malawi
Shrubby Pigeon Peas Transform Malawi Farming: 1st-Generation Perennial Grain Legumes

Sieglinde S. Snapp, Michigan State University, Hickory Corners
Next Steps and Research Needs in Perennial Grain Development

Andrew Paterson, University of Georgia, Athens
Genomic Innovations for Next-Generation Perennial Grain Crops

Symposium Tracks

Agricultural, Plant, and Food Sciences

A Changing Global Landscape: Evolving Roles of BRIC Nations in Agricultural Sciences

Organized by Rodney A. Hill, University of Idaho, Moscow

Biosciences for Farming in Africa

Organized by David J. Bennett, St. Edmund's College, Cambridge, United Kingdom

Innovative and Integrated Approaches To Reducing Malnutrition

Organized by Jennifer Long and Ahmed Kablan, USAID, Washington, DC

Securing Food, Feed, and Fuel via Natural Diversity: Spotlight on the Maize Genome

Organized by Patrick Regan, Ulrich Marsch, and Barbara Wankerl, Technical University Munich, Germany

Anthropology, Culture, and Language

Comparative Advantage: Global Perspectives on Human Biology and Health

Organized by Thomas McDade and William Leonard, Northwestern University, Evanston, IL

Neoracism and Scientific Racism in "Post-Racial" Societies

Organized by Nina Jablonski, Pennsylvania State University, University Park; Robert W. Sussman, Washington University, St. Louis, MO

Preserving Our Cultural Heritage: Science in the Service of Art

Organized by Leonor Sierra and Nicholas Bigelow, University of Rochester, NY

Reconstructing and Deconstructing Paintings: Innovations At and Below the Surface

Organized by Francesca Casadio, The Art Institute of Chicago, IL; Katherine Faber, Northwestern University, Evanston, IL

Rethinking Repatriation of Human Remains: Is It Possible to Move Beyond Conflict?

Organized by Norman MacLeod and Margaret Clegg, Natural History Museum, London, United Kingdom

Talking to Kids Really Matters: Early Language Experience Shapes Later Life Chances

Organized by Anne Fernald, Stanford University, CA

The Large Cognitive Implications of Small Languages

Organized by D. H. Whalen, City University of New York, New York City

Variability in Speech and Language in Individuals with Autism and Associated Traits

Organized by Alan C. Yu, University of Chicago, IL

Behavioral and Social Sciences

Building Babies: Development, Evolution, and Human Health

Organized by Katie Hinde, Harvard University, Cambridge, MA

Guns and Violence: Psychological, Economic, Political, and Public Policy Implications

Organized by Martin S. Banks, University of California, Berkeley; Garen J. Wintemute, University of California, Davis; Richard N. Aslin, University of Rochester, NY

How to Rebuild Informed Trust in Science: Insights from Social Sciences

Organized by Rainer Bromme, University of Muenster, Germany

Learning about People and Society via Analysis of Large-Scale Data on Human Activities

Organized by Eric Horvitz, Microsoft Research, Redmond, WA

Physiological and Cultural Foundations of Human Social Behavior

Organized by Geraldine Barry, European Commission, Joint Research Center, Brussels, Belgium

Project Teams and Public Expenditures of Scientific Research: An International Comparison

Organized by Julia Lane, American Institutes for Research, Washington, DC

Rhythmic Entrainment in Non-Human Animals: An Evolutionary Trail of Time Perception

Organized by Patricia M. Gray, University of North Carolina, Greensboro, NC

The Science of Resilient Aging

Organized by Elizabeth A. L. Stine-Morrow, University of Illinois, Urbana-Champaign

Using Social Science to Change Decisions and Improve Health Outcomes

Organized by Arthur Lupia, University of Michigan, Ann Arbor

Biology and Neuroscience

Addiction: Our Compulsions and Brain Reward Systems

Organized by Wilson Compton, National Institute on Drug Abuse, Bethesda, MD; Aidan Gilligan, SciCom—Making Sense of Science, Brussels, Belgium

Epigenetic Control of Brain and Behavior

Organized by Joseph Coyle, Harvard Medical School, Belmont, MA

Genetic and Epigenetic Determinants of Susceptibility to Toxicants

Organized by Berran Yucesoy, National Institute for Occupational Safety and Health, Morgantown, WV; Victor J. Johnson, Burleson Research Technologies Inc., Morrisville, NC

Intelligent Autonomous Robots: Biologically Inspired Engineering

Organized by John G. Hildebrand, University of Arizona, Tucson

Inventing New Ways To Understand the Human Brain

Organized by Hillary Sanctuary and Richard Walker, Swiss Federal Institute of Technology (EPFL), Lausanne; Megan Williams, Swissnex, San Francisco, CA

Molecular Basis of Age-Related Susceptibility to Chemicals and Environmental Hazards

Organized by Janice S. Lee, U.S. Environmental Protection Agency (EPA), Research Triangle Park, NC

New Insights into Animal Behavior: The Role of the Microbiome

Organized by Vanessa Ezenwa, University of Georgia, Athens; Daniel Rubenstein, Princeton University, NJ; Joy Bergelson, University of Chicago, IL

Non-Coding RNA in Development and Disease

Organized by Gary Felsenfeld, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD; Jeannie T. Lee, Massachusetts General Hospital, Boston

Solitary Confinement: Legal, Clinical, and Neurobiological Perspectives

Organized by Michael J. Zigmond, University of Pittsburgh, PA

Synthetic Biology Approaches to New Chemistry

Organized by Michelle C. Chang and Jay D. Keasling, University of California, Berkeley

Video Games, Brains, and Society

Organized by Susan Hagen and Daphne Bavelier, University of Rochester, NY

Your Genome: To Share or Not To Share?

Organized by Yaniv Erlich, Whitehead Institute for Biomedical Research, Cambridge, MA

Communication and Public Programs

Improvisation for Scientists: Making a Human Connection

Organized by Valeri Lantz-Gefroh and Elizabeth Bass, Stony Brook University, NY

Innovative Vehicles for Vetted Information in a Wiki World

Organized by Sarah Bates, Society for Neuroscience, Washington, DC

Religious Communities, Science, Scientists, and Perceptions: A Comprehensive Survey

Organized by Paul Arveson and Jennifer Wiseman, AAAS Center for Science, Policy, and Society Programs, Washington, DC

Science Festivals as Regional Collaborations: Extending Resources by Working Together

Organized by Ben Wiehe, MIT Museum, Cambridge, MA

Science, Religion, and Modern Physicists: New Studies

Organized by Paul Arveson and Jennifer Wiseman, AAAS Center for Science, Policy, and Society Programs, Washington, DC

Securing the Future of Science: Using the Higgs to Inspire the Young

Organized by Timothy Meyer, TRIUMF, Vancouver, Canada; Terry O'Connor, Science and Technology Facilities Council, Swindon, United Kingdom

Stakeholder Engagement in Science: Strategies, Experiences, and Implications

Organized by Samantha J. Jones and Louis J. D'Amico, EPA, Washington, DC

Teen Cafés: Innovative Model for Effective Science Communication with Key Demographic

Organized by Michelle Hall, Science Education Solutions Inc., Los Alamos, NM

What Do People Think about Science and Technology? U.S. and International Public Opinion

Organized by John C. Besley, Michigan State University, East Lansing

Where's My Flying Car? Science, Science Fiction, and a Changing Vision of the Future

Organized by Susan Wolfenbarger and Jonathan Drake, AAAS Center for Science, Policy, and Society Programs, Washington, DC

Computer Science, Mathematics, and Statistics

Advances in Citizen Science: Large-Scale Community Engagement for Sensing and Analysis

Organized by Eric Horvitz, Microsoft Research, Redmond, WA Elections Through the Lens of Mathematics

Organized by D. Marc Kilgour, Wilfrid Laurier University, Waterloo, Canada

Intelligent Context-Aware Systems for Healthcare, Wellness, and Assisted Living

Organized by Louise Byrne, European Commission, Research Executive Agency, Brussels, Belgium

Outsourcing Science: Will the Cloud Transform Research?

Organized by Ian Foster, Argonne National Laboratory, IL

People and Computing: On Human-Computer Collaborations for Tackling Hard Problems

Organized by Erwin P. Gianchandani, National Science Foundation (NSF), Arlington, VA; Eric Horvitz, Microsoft Research, Redmond, WA

Re-Identification Risk of De-Identified Data Sets in the Era of Big Data

Organized by Xiao Hua Andrew Zhou, University of Washington, Seattle; Leslie Taylor, VA Puget Sound Health Care System, Seattle, WA

Statistical Methods for Large Environmental Datasets

Organized by Charmaine Dean, University of Western Ontario, London, Canada

The Importance of Recreational Mathematics in Solving Practical Problems

Organized by Laura Taalman and Jason Rosenhouse, James Madison University, Harrisonburg, VA

Virtual Humans: Helping Facilitate Breakthroughs in Medicine

Organized by Ram D. Sriram, National Institute of Standards and Technology, Gaithersburg, MD; Ramesh Jain, University of California, Irvine; Donald Henson, George Washington University, Washington, DC

Education and Human Resources

Analogical Processes in STEM Learning

Organized by Dedre Gentner, Northwestern University, Evanston, IL

Beyond the Pipeline: New Strategies to Build a Competitive and Diverse Workforce

Organized by Kenneth Gibbs, NSF, Arlington, VA

Building National Capacity in Science Communication for STEM Graduate Students

Organized by Erica Goldman and Elizabeth Neeley, Communication Partnership for Science and the Sea, Silver Spring, MD

Creating an Ecosystem for Science Learning In and Out of School

Organized by Dennis Schatz, NSF, Arlington, VA; Martin Storksdieck, National Research Council, Washington, DC

Leveling the Playing Field: Why Cultural Relevance Matters in Computer Science

Organized by Legand Burge and Alicia N. Washington, Howard University, Washington, DC

Rebooting Our Approach to Increasing Indigenous STEM Participation: Lessons from Hawaii

Organized by Timothy F. Slater, University of Wyoming, Laramie

STEM Education Policies and Policymaking: Pushing in the Same Direction

Organized by Catherine Middlecamp, University of Wisconsin, Madison; Judith A. Ramaley, Portland State University, OR

The Central Role of Energy Concepts in K-12 Science Education

Organized by Arthur Eisenkraft, University of Massachusetts, Boston

Thinking Skills for the 21st Century: Teaching for Transfer

Organized by Eleanor V.H. Vandegrift, University of Oregon, Eugene; Amy B. Mulnix, Earlham College, Richmond, IN

Use of Digital Games To Support Youth's Engagement with Science and Technology

Organized by Patricia L. Ward, Museum of Science and Industry, Chicago, IL

Women Poised for Discovery and Innovation: Resolving the Remaining Hurdles

Organized by Lynnette D. Madsen, NSF, Arlington, VA; Catherine Mavriplis, University of Ottawa, Canada

Energy and Renewable Resources

Chemistry and Materials Science of Solar Energy Utilization

Organized by John Rogers, University of Illinois, Urbana-Champaign

Hydraulic Fracturing: Science, Technology, Myths, and Challenges

Organized by Christopher B. Harto and Alfred P. Sattelberger, Argonne National Laboratory, IL

Is It Possible to Reduce 80% of Greenhouse Gas Emissions from Energy by 2050?

Organized by Jane C.S. Long, Lawrence Livermore National Laboratory, CA; Steve Hamburg, Environmental Defense Fund, Washington, DC; Armond Cohen, Clean Air Task Force, Boston, MA

Making Power, Taking Power: Renewable Microgrids in National Electricity Strategies

Organized by Michael Isaacson, University of California, Santa Cruz

Nanoelectronics for Renewable Energy: How Nanoscale Innovations Address Global Needs

Organized by William Gilroy, University of Notre Dame, IN; Hillary Sanctuary, EPFL, Lausanne, Switzerland; Patrick Regan, Technical University Munich, Germany

Next-Generation Electrical Energy Storage: Beyond Lithium Ion Batteries

Organized by Jeff Chamberlain and George Crabtree, Argonne National Laboratory, IL

Opportunities and Challenges for Nuclear Small Modular Reactors

Organized by Granger Morgan, Carnegie Mellon University, Pittsburgh, PA; Elisabeth A. Gilmore, University of Maryland, College Park

Engineering, Industry and Technology

U.S. National User Facilities: A Major Force for Discovery and Innovation

Organized by Susan Strasser, Argonne National Laboratory, IL; Ben Brown, U.S. Department of Energy, Washington, DC

Discovery and Innovation in Science and Engineering Security Technologies

Organized by Anice Anderson, Private Engineering Consulting, Carmel, IN; Benn Tannenbaum, Sandia National Laboratories, Washington, DC; Cammy Abernathy, University of Florida, Gainesville

Emergency Response and Community Resilience via Engineering and Computational Advances

Organized by Eva Lee, Georgia Institute of Technology, Atlanta

Innovation in Community Deployment of Water Technologies

Organized by Sushanta Mitra and Thomas Thundat, University of Alberta, Edmonton, Canada

Innovations in Crystallography Meet Demands in Materials Science, Energy, and Health

Organized by Tona Kunz, Argonne National Laboratory, IL

Integrated Cellular Systems: Building Machines with Cells

Organized by Nicholas A. Peppas, University of Texas, Austin; Rashid Bashir, University of Illinois, Urbana-Champaign

Open Science: Reducing Barriers to Scientific Breakthroughs

Organized by Kathryn L. Lovero, University of California, San Francisco; Lina Nilsson and Todd A. Duncombe, University of California, Berkeley

The Future of Cities: Dense or Dispersed?

Organized by Antony Wood, Council on Tall Buildings and Urban Habitat, Chicago, IL; Daniel Safarik and John Ronan, Illinois Institute of Technology, Chicago

Unlocking the Power of Big Data by Integrating Physical, Engineering, and Life Sciences

Organized by Sean E. Hanlon, National Cancer Institute, Bethesda, MD

Environment and Ecology

Agrobiodiversity and Global Change: New Linkages to Sustainability

Organized by Karl Zimmerer, Pennsylvania State University, State College

Canada's Oil Sands: Environmental and Economic Dimensions

Organized by Amir Mokhtari Fard, Southern Alberta Institute of Technology, Calgary, Canada

Changing Earth and Eco Systems in the Antarctic Peninsula

Organized by Eugene W. Domack, University of South Florida, St. Petersburg; Jere H. Lipps, California State University, Fullerton

Discovering Long-Term Climate Vulnerabilities at the Nature-Society Interface

Organized by Christopher I. Roos, Southern Methodist University, Dallas, TX

Earth Observation Data Goes Open Access

Organized by Gilles Ollier, European Commission, Directorate General for Research and Innovation, Brussels, Belgium

Research Challenges in Climate Change: What's New and Where Are We Going?

Organized by Thomas R. Karl, NOAA, Asheville, NC; Jerry Melillo, Marine Biology Laboratory, Woods Hole, MA; Donald J. Wuebbles, University of Illinois, Urbana-Champaign

Santa's Revenge: The Impacts of Arctic Warming on the Mid-Latitudes

Organized by Michael MacCracken, Climate Institute, Washington, DC; Ester Szein, U.S. National Academies, Washington, DC

The Arctic Cocktail: Shaken, Not Stirred

Organized by Franz Immler, European Commission, Directorate General for Research and Innovation, Brussels, Belgium

The Big Thaw: Impacts on Health of Marine Mammals and Indigenous People in the Arctic

Organized by Andrew Trites, North Pacific Universities Marine Mammal Research Consortium, Vancouver, Canada; Stephen A. Raverty, British Columbia Ministry of Agriculture and Lands, Abbotsford, Canada; Mike E. Grigg, National Institutes of Health, Bethesda, MD

The Evolving Great Lakes: New Techniques, Discoveries, and Management Implications

Organized by Thomas C. Johnson, University of Minnesota, Duluth; Robert E. Hecky, Large Lakes Observatory, Duluth, MN

Global Perspectives and Issues

Accelerating Innovation in the Middle East: Lessons for the Developing World

Organized by Lara Campbell, CUBRC Center for International Science and Technology Advancement, Washington, DC

Building Global Partnerships: Sharing Discovery and Innovation, Safeguarding Difference

Organized by Aidan Gilligan, SciCom-Making Sense of Science, Brussels, Belgium; Daan Du Toit, South African Department of Science and Technology, Brussels, Belgium

Challenges in Conducting Risk Based Technology Assessments Globally

Organized by Umesh Thakkar, U.S. Government Accountability Office, Washington, DC

Competing Universities Collaborate on Standard Metrics for Global Benchmarking

Organized by John Green, Snowball Metrics Steering Committee, Cambridge, United Kingdom

Evaluating the Global Impact of Research Investments

Organized by Shannon L. Griswold and David J. Proctor, NSF, Arlington, VA; Kristina Wagstrom, University of Connecticut, Storrs

Focusing the Gender Lens on Science and Innovation: Improving Lives and Livelihoods

Organized by Sophia Huyer, Organization for Women in Science for the Developing World, Brighton, Canada

Globally Shipped But What's in the Box? Innovation for Better Container Security

Organized by Stephan Lechner, European Commission, Joint Research Center, Ispra, Italy

Grand Challenges: Science and Technology Solutions for International Development

Organized by Ku McMahan, USAID, Washington, DC

Innovation and Collaboration at 17,500 MPH: The International Space Station Experience

Organized by Kirt A. Costello, National Aeronautics and Space Administration, Houston, TX

Innovation in Global Health Research: Bridging the Knowledge-to-Action Divide

Organized by Emma Cohen, Canadian Institutes of Health Research, Ottawa, Canada

Resolving Our Greatest Public Health Challenges via Science Diplomacy

Organized by Michel Kazatchkine, United Nations, Geneva, Switzerland; Aidan Gilligan, SciCom-Making Sense of Science, Brussels, Belgium

Risk-Based Standards for Cybersecurity: Global Challenges and Solutions

Organized by Elke Anklam, European Commission, Joint Research Center, Geel, Belgium; Igor Linkov, U.S. Army Engineer Research and Development Center, Concord, MA

Web-Based Technologies Change International Research Collaborations

Organized by Stefania Di Mauro-Nava, CRDF Global, Arlington, VA

Innovation and Entrepreneurship

Understanding the Science Needed for Sustainable Urban Development

Organized by Jan Riise, European Science Events Association, Onsala, Sweden

Convergence Science: A Revolution for Health Solutions

Organized by Joseph M. DeSimone, University of North Carolina, Chapel Hill; Amanda J. Arnold and Maggie Lloyd, Massachusetts Institute of Technology, Cambridge

Leveraging Resources, Organization, and Collaboration for Breakthrough Science

Organized by Philip Shapira, University of Manchester, United Kingdom; Jerald Hage, University of Maryland, College Park

Making the Best Use of Academic Knowledge in Innovation Systems

Organized by Koichi Sumikura, Taro Matsubara, and Aska Takeshiro, National Institute of Science and Technology Policy, Tokyo, Japan

New North-South Funding for Fighting Poverty-Related Diseases

Organized by Line Matthiessen, European Commission, Brussels, Belgium

Nurturing Scientific Innovation and Entrepreneurship within the University Ecosystem

Organized by Phil Weilerstein, National Collegiate Inventors and Innovators Alliance, Hadley, MA

U.S. Looks to the Global Science, Technology, and Innovation Horizon

Organized by Elizabeth E. Lyons, U.S. Department of State, Washington, DC

Medical Sciences and Public Health

48 Hours To Save the World: Challenge of the Next Pandemic

Organized by Line Matthiessen, European Commission, Brussels, Belgium

Air Pollution as a Risk Factor for Central Nervous System Diseases and Disorders

Organized by Deborah A. Cory-Slechta, University of Rochester, NY; Michelle L. Block, Virginia Commonwealth University, Richmond

Approaches for Ensuring Children's Environmental Health Protection

Organized by Sally P. Darney, EPA, Research Triangle Park, NC

Artificial Tissues Engineered To Improve Patient Well-being

Organized by Louise Byrne, European Commission, Research Executive Agency, Brussels, Belgium

Bio-Surveillance: The Interface of Biological, Physical, and Information Sciences

Organized by Basil I. Swanson, Los Alamos National Laboratory, NM

Global Public Health Security: It Takes a Village

Organized by David Blazes, Johns Hopkins University, Baltimore, MD

Harnessing the Immune System: From Bench to Bedside and Back Again

Organized by Angela C. Colmone, AAAS/Science Translational Medicine, Washington, DC

How Does Oral Health Fit in the Emerging Health Care Environment?

Organized by Paul H. Krebsbach and Peter J. Polverini, University of Michigan, Ann Arbor

Inside Out: The Impact of Gut Flora on Diabetes and Obesity

Organized by Isabelle Kling, European Molecular Biology Laboratory, Heidelberg, Germany

Nexus of Cell Signaling and Drug Therapy: Oxygen, Phosphorus, Sulfur, and Nitrogen

Organized by Kenneth D. Tew, Medical University of South Carolina, Charleston

Physics and Astronomy

Dark Matter Discoveries: Challenges and Innovation

Organized by Maria Spiropulu, California Institute of Technology, Pasadena

Exploring the Foundations of Magnetism with New Nanoscale Probes

Organized by Michael E. Flatté, University of Iowa, Iowa City

Extremities of the Cosmos: New Experimental Results in Particle Astrophysics

Organized by Craig Hogan, Fermilab and University of Chicago, IL

From Dust and Gas to Disks and Planets

Organized by Mark T. Adams, National Radio Astronomy Observatory, Charlottesville, VA

New Millimeter-Wavelength Insights into Galaxy Evolution in the Early Universe

Organized by Mark T. Adams, National Radio Astronomy Observatory, Charlottesville, VA

Optics and Photonics: An International Perspective

Organized by Erik B. Svedberg, U.S. National Academies, Washington, DC; Alan Eli Willner, University of Southern California, Los Angeles; Paul McManamon, Exciting Technology, LLC, Dayton, OH

Quantum Information Technologies

Organized by Martin Laforest, University of Waterloo, Canada

Stars in the Laboratory: Fundamental Nuclear Physics at the National Ignition Facility

Organized by Ani Aprahamian, University of Notre Dame, IN; Elizabeth R. Cantwell and Christopher J. Keane, Lawrence Livermore National Laboratory, CA

Targeting Tumors: Ion Beam Accelerators Take Aim at Cancer

Organized by Karen McNulty Walsh and Stephen Peggs, Brookhaven National Laboratory, Upton, NY; James Siegrist, U.S. Department of Energy, Washington, DC

Technological Innovations and Their Impact on Astronomical Discovery

Organized by Donald Campbell, Cornell University, Ithaca, NY; Margaret Meixner, Space Telescope Science Institute, Baltimore, MD

The Physics of Information

Organized by Michel Devoret and Norman Chonacky, Yale University, New Haven, CT

Public Policy

Air Quality and Climate Change: Science and Policy Challenges

Organized by Julia Schmale and Erika von Schneidemesser, Institute for Advanced Sustainability Studies, Potsdam, Germany

Decision-Making in the Public Domain: Boundary Processes as Catalysts for Innovation

Organized by Steven Courtney, RESOLVE, Washington, DC

Discovery and Innovation: What's the Connection and Why Does It Matter?

Organized by Jason S. Robert, Arizona State University, Tempe

Global Excellence: New Drivers and Innovative Solutions

Organized by David Budtz Pedersen and Klaus Bock, Danish Ministry of Science, Technology and Innovation, Copenhagen

Responsible Innovation in a Global Context

Organized by David H. Guston, Arizona State University, Tempe

Scholarly Publishing Innovations and Evolution: Views of the Stakeholders

Organized by H. Frederick Dylla, American Institute of Physics, College Park, MD

Science Policy-Making that Meets Social Challenges and Motivates Scientists

Organized by Tateo Arimoto, National Graduate School for Policy Studies, Tokyo, Japan; Chikako Maeda, Japan Science and Technology Agency, Tokyo

Streamlining U.S. Visa and Immigration Policies

Organized by Amy Flatten, American Physical Society, College Park, MD; Albert H. Teich, George Washington University, Washington, DC

The Golden Goose Award: Highlighting the Value of Federal Support for Basic Research

Organized by Tobin L. Smith and Julia Smith, Association of American Universities, Washington, DC; Jennifer Poulakidas, Association of Public and Land-grant Universities, Washington, DC

Transplant Organ Shortage: Informing National Policies Using Management Sciences

Organized by Michael Abecassis and Sanjay Mehrotra, Northwestern University, Evanston, IL

Will the Workplace of Tomorrow Have Any Workers? Computing, Productivity, and Jobs

Organized by David H. Autor, Massachusetts Institute of Technology, Cambridge

Sustainability and Resource Management

Challenges and Opportunities in Transitioning Small-Scale Fisheries to Sustainability

Organized by Elena M. Finkbeiner and Larry Crowder, Stanford University, CA

Deep-Ocean Industrialization: A New Stewardship Frontier

Organized by Lisa Levin, Scripps Institution of Oceanography, La Jolla, CA; Kristina Gjerde, International Union for Conservation of Nature, Konstancin-Chylce, Poland

Hazards: What Do We Build For?

Organized by Julia R. Wilson, Sense About Science, London, United Kingdom

Making Products Sustainable as Materials Become Scarce

Organized by Erno Vandeweert and Aud Helen Alming, European Commission, Brussels, Belgium

New Modeling Approaches to Inform Climate Change Understanding and Decision-Making

Organized by Thomas Dietz, Michigan State University, East Lansing

New Scenarios for Assessing Future Climate Change

Organized by Peter Backlund and Brian C. O'Neill, National Center for Atmospheric Research, Boulder, CO

Securing Fisheries Through Novel Approaches: Opportunities of Genetics and Genomics

Organized by Geraldine Barry, European Commission, Joint Research Center, Brussels, Belgium

Systems Innovation Experience (SIX) in Materials for Sustainable Development

Organized by Alan Hurd, U.S. Department of State, Washington, DC

The Ocean Tracking Network: Global Innovation in Technology, Science, and Management

Organized by Frederick G. Whoriskey and Sara J. Iverson, Dalhousie University, Halifax, Canada

The Soils of Africa: A Forgotten Resource

Organized by Luca Montanarella, European Commission, Joint Research Center, Ispra, Italy; Geraldine Barry, European Commission, Joint Research Center, Brussels, Belgium

AAAS, publisher of *Science*, thanks the sponsors and supporters of the 2014 Annual Meeting



AAAS thanks



for its generous support of the Science Journalism Awards

AAAS|2014
ANNUAL MEETING
13-17 FEBRUARY • CHICAGO

Register Today at Discounted Rates

REGISTRATION

Discounted advance registration rates are available until 22 January 2014.
For more information, visit www.aaas.org/meetings

	Member	New Member	Non-Member
Professional	\$295	\$380	\$399
Postdoc	\$235	\$320	\$335
K-12 Teacher	\$235	\$320	\$335
Emeritus	\$235	\$320	\$335
Student	\$60	\$70	\$90

HOTELS

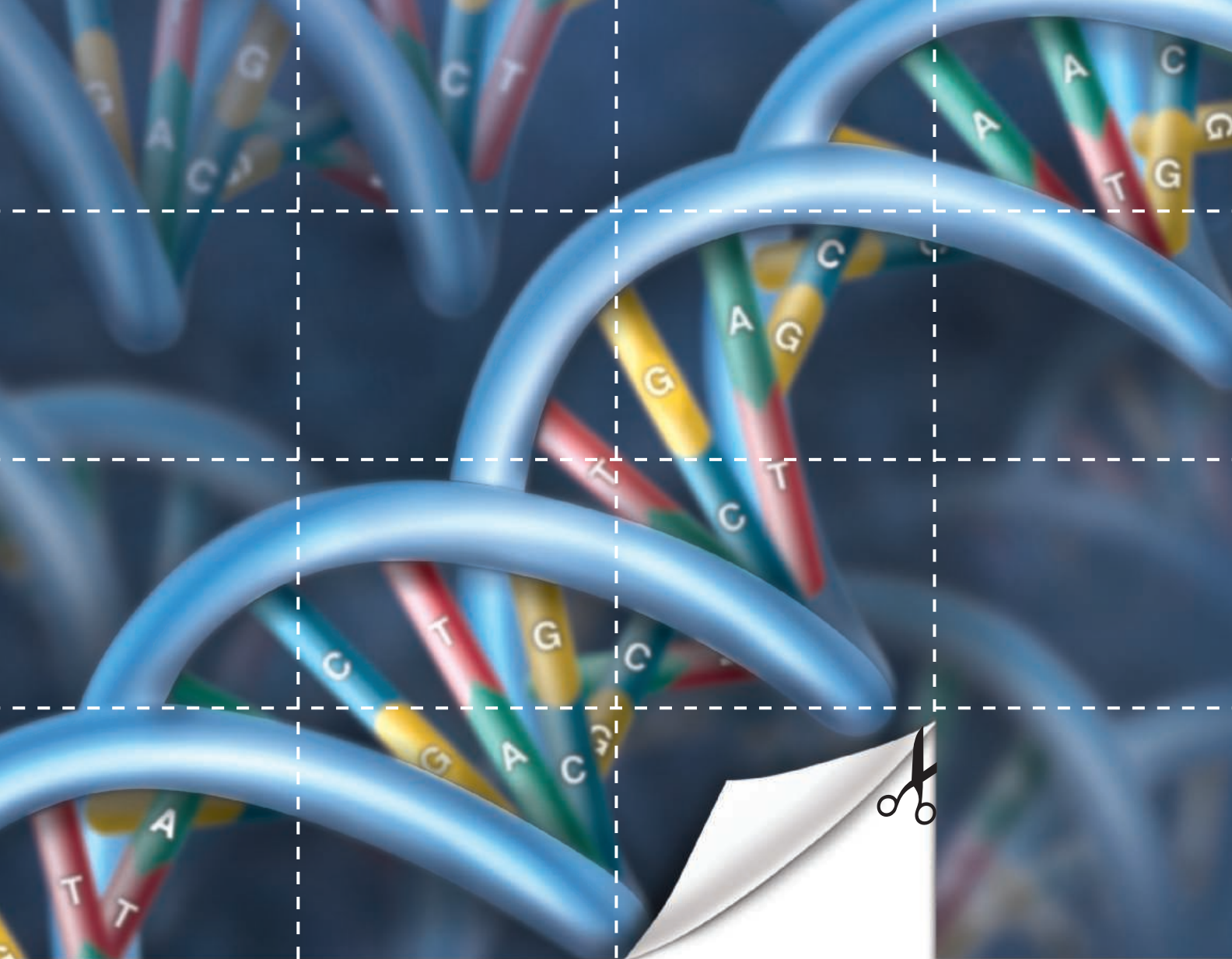
Special room rates and travel benefits are available to Annual Meeting registrants.

Hyatt Regency Chicago
Rate: \$199 single/double

Fairmont Chicago
Rate: \$168 single/double

Swissôtel Chicago
Rate: \$156 single/\$176 double

Rooms are available on a first-come, first-served basis until 22 January 2014.



Cut smarter.

Restriction enzymes from NEB –
now with CutSmart™ Buffer

You make smart choices every day. Why stop there? The choice to use restriction enzymes from New England Biolabs is now even easier.

- Choose from > 200 restriction enzymes supplied with a single buffer
- Simplify your double digest reactions
- Reduce your pipetting steps by no longer having to add BSA

Now, that's Smart!



Explore the smarter choice at
NEBCutSmart.com

 NEW ENGLAND
BioLabs Inc.
enabling technologies in the life sciences



Science Careers Advertising

For full advertising details, go to ScienceCareers.org and click For Employers, or call one of our representatives.

Tracy Holmes
Worldwide Associate Director
Science Careers
Phone: +44 (0) 1223 326525

THE AMERICAS

E-mail: advertise@sciencecareers.org
Fax: 202-289-6742

Tina Burks
East Coast/West Coast/South America
Phone: 202-326-6577

Marci Gallun
Midwest/Canada
Phone: 202-326-6582

Candice Nulsen
Corporate
Phone: 202-256-1528

Online Job Posting Questions
Phone: 202-312-6375

EUROPE / INDIA / AUSTRALIA / NEW ZEALAND / REST OF WORLD

E-mail: ads@science-int.co.uk
Fax: +44 (0) 1223 326532

Axel Gesatzki
Phone: +44 (0)1223 326529

Sarah Lelarge
Phone: +44 (0) 1223 326527

Kelly Grace
Phone: +44 (0) 1223 326528

JAPAN

Yuri Kobayashi
Phone: +81-(0)90-9110-1719
E-mail: ykobayas@aaas.org

CHINA / KOREA / SINGAPORE / TAIWAN / THAILAND

Ruolei Wu
Phone: +86-1367-1015-294
E-mail: rwu@aaas.org

All ads submitted for publication must comply with applicable U.S. and non-U.S. laws. *Science* reserves the right to refuse any advertisement at its sole discretion for any reason, including without limitation for offensive language or inappropriate content, and all advertising is subject to publisher approval. *Science* encourages our readers to alert us to any ads that they feel may be discriminatory or offensive.

Science Careers

From the journal *Science*



ScienceCareers.org

ScienceCareers.org

We know science



The American Association for the Advancement of Science (AAAS) seeks a Writer II to write and edit articles for *Science* Careers website.

Major duties and responsibilities:

- Report, write, and edit features and blog postings for Science Careers
- Stay abreast of news, information, and issues related to science careers via press releases, personal contacts, and other sources.
- Work with the *Science* Careers editor to commission articles
- Work with writers and editors to secure art for Science Careers articles
- Attend *Science* news department meetings and meetings with *Science* Business Office and AAAS units related to science careers
- Occasionally write career-related news for Science magazine and its online news sites

Minimum qualifications:

- Extensive university or college level training leading to at least a Bachelor's degree (some graduate studies and research preferred)
- At least three to five years of professional experience in science writing, reporting and editing
- Demonstrated written and verbal communication skills
- Demonstrated knowledge of science-related issues and research
- Familiarity with social networking and blogging platforms
- Ability to work as part of a widely dispersed but close-knit team
- Ability to communicate with public, professional, and diverse audiences
- Strong computer skills
- Experience writing about science career issues preferred

Please visit our website:

<http://www.aaas.org/careercenter/employmentataaas/>
to get more information and to apply to AAAS online.

AAAS is an Equal Opportunity Employer.

Top Employers Survey 2013: Top Firms Directed by Data, Led by Scientists

Developing pharmaceuticals requires huge investments of time, human resources, and capital. The companies identified in the 2013 *Science* Careers Top Employers Survey ensure a higher return on those investments by catering to the whims of the scientist brain, which they view as their greatest economic driver. These employers give scientists the intellectual time and space to dream up novel ways of blocking, shutting down, or modifying disease targets. They marry the academic freedom found in the university hallway to powerhouse financial resources and technological platforms to get research done at a quicker pace. That combination results in researchers who are not only satisfied in their jobs, but also successful at creating new drugs. **By Kendall Powell**



Although the overall economic outlook of the biotechnology and pharmaceutical sectors has remained strong through the recent gloomy financial times, there remain significant challenges ahead for the industry. According to the Pharmaceutical Research and Manufacturers of America (PhRMA), companies need on average 10–15 years and more than \$1.2 billion to develop a drug. And a coming wave of blockbuster drug patent expirations is expected to cost the industry tens of billions in lost revenues. PhRMA reports that 84 percent of all prescriptions are now for generic drugs, up from 49 percent in 2000.

How do top employers rise above these considerable hurdles? Above all, by placing science squarely at the center of their organizations and scientists at the top rungs of leadership. At these workplaces, the data drive decisions and project directions, which largely puts scientists in the drivers' seats.

"The whole stream of innovation and the speed with which you can take an idea and get a drug candidate ready for the clinic gets people jazzed up about working here," says **Neil Stahl**, senior vice president of research and development sciences at Regeneron Pharmaceuticals, Inc., which is #1 on the 2013 *Science* Careers Top Employers Survey for a second year in a row. Stahl notes that the biotechnology company took its monoclonal antibody alirocumab for lowering cholesterol from concept to clinical trials in just 19 months. The Tarrytown, New York-based biotechnology firm has made its reputation on innovations that bust the bottlenecks of drug discovery and development.

"By far, my favorite thing about working here is to get into a room with a glimmer of an idea, and by the time you leave, you have something that could turn into the next great technology," says Stahl.

Even though tough economic realities face Eli Lilly and Company, Lilly's commitment to long-term investment in research has given

it the highest ranking among the largest, in terms of revenue, pharmaceutical firms, at #5. Other smaller companies like Genentech (#2, up from #3 in 2012), Regeneron, Biocon Limited (#6, up from #19), and Gilead Sciences (#15, up from #18) are also focused on the long-term, hiring research personnel and continuing to invest heavily in research and development.

The secrets to these companies' success lie in giving employees the flexibility to manage their own time and schedules, listening to their good ideas, and letting go of the concept of "failure." Top employers also hold a clear vision that keeps scientists motivated and working on the same page, even when they may have strong intellectual differences of opinion. That is aided by consistently strong scientific leadership at the top-most rungs of the organization. And finally, these corporations give their employees opportunities to take ample breaks from their intense work to regroup and return to their pursuits refreshed.

"We have a culture of recognition and celebration. We take a break and dance around," says **Ann Lee-Karlon**, senior vice president of portfolio management and operations for Genentech Research and Early Development, based in South San Francisco, referring to celebrations like the annual Give Back concert for Genentech employees. Like other top employers, her firm provides a few unusual benefits designed to help employees focus on their work with minimal stress about daily life—such as on-site dry cleaning, car washes, haircuts, and concierge services. Other life-easing perks include company vans to commute across the notorious Bangalore traffic (Biocon), pet insurance and family flu shots (Regeneron), and backup day- or eldercare (Gilead).

An undercurrent of fun—being able to play hard alongside hard work—attracts top talent to Genentech, which has never dipped below a rank of #3 in the entire 12-year history of the survey. "There's something really great about finding a place to do meaningful work and be recognized for it," says Lee-Karlon. "That's really joyful."

Upcoming Features

Neuroscience Careers—November 1
Regional Focus: China—November 15
Faculty Careers—February 7, 2014

How a Top Employer is Built

Each year, *Science* commissions a survey to identify the top employers in the biotechnology and pharmaceutical industry and to determine the characteristics upon which scientists base their rankings. This year, the results are based on 3,656 responses to a web-based survey deployed by e-mail (see Survey Methodology in chart, page 498).

continued>

Top Twenty Employers

2013 Rank 2012 Rank Employer (Global Headquarters)

2013 Rank	2012 Rank	Employer (Global Headquarters)	Innovative leader in the industry	Treats employees with respect	Is socially responsible	Has loyal employees	Has a clear vision	Does important, quality research
1	1	Regeneron Pharmaceuticals, Inc. (Tarrytown, NY)	•	•	•	•	•	•
2	3	Genentech (South San Francisco, CA)	•	•	•	•	•	•
3	2	Vertex Pharmaceuticals Incorporated (Cambridge, MA)	•	•	•	•	•	•
4	–	AbbVie (North Chicago, IL)	•	•	•	•	•	•
5	–	Eli Lilly and Company (Indianapolis, IN)	•	•	•	•	•	•
6	19	Biocon Limited (Bengaluru, Karnataka, India)	•	•	•	•	•	•
7	6	Millennium: The Takeda Oncology Company (Cambridge, MA)	•	•	•	•	•	•
8	11	Novartis (Basel, Switzerland)	•	•	•	•	•	•
9	7	Boehringer Ingelheim (Ingelheim, Germany)	•	•	•	•	•	•
10	9	Biogen Idec (Weston, MA)	•	•	•	•	•	•
11	4	Novo Nordisk (Bagsvaerd, Denmark)	•	•	•	•	•	•
12	10	DuPont (Wilmington, DE)	•	•	•	•	•	•
13	17	Syngenta (Basel, Switzerland)	•	•	•	•	•	•
14	5	Monsanto Company (Creve Coeur, MO)	•	•	•	•	•	•
15	18	Gilead Sciences (Foster City, CA)	•	•	•	•	•	•
16	8	Roche—excluding Genentech (Basel, Switzerland)	•	•	•	•	•	•
17	12	Celgene (Summit, NJ)	•	•	•	•	•	•
18	15	Abbott (Abbott Park, IL)	•	•	•	•	•	•
19	13	Amgen (Thousand Oaks, CA)	•	•	•	•	•	•
20	20	Bayer (Leverkusen, Germany)	•	•	•	•	•	•



Survey Methodology

Science Careers conducted its annual web-based survey of individuals familiar with biotechnology and pharmaceutical employers to determine the best employers in the field. This survey was conducted from March 5 to March 31, 2013. Roughly 25,000 individuals from the AAAS database and former survey participants were invited via e-mail messages to take the survey. Individuals working in human resources at biotech and pharma companies (Science Careers sales database) were also contacted by e-mail to promote the survey within their organization.

In all, 3,656 surveys were submitted, which served as the basis for the analysis. The top 20 companies were selected using a statistical process that calculates a unique ranking score for each company rated. Only companies rated by 30 or more respondents were eligible to become part of the top 20 best employers.

The 20 companies with the best reputations as employers and the top three driving characteristics for each individual company, according to respondents in the 2013 survey undertaken for the Science/AAAS Custom Publishing Office. The companies without a 2012 rank did not receive enough mentions to qualify or did not receive a high enough ranking during the 2012 survey.

The vast majority of respondents (75 percent) report not yet reaching the peak of their career, but almost two-thirds (65 percent) have been in the workforce for at least 10 years. Basic researchers made up 19 percent of the survey respondents, while 25 percent work in applied research, 25 percent work in development, and 10 percent are administrators or executives (see Survey Demographics box, page 500). This year, of the 21 percent of respondents who said they were likely to seek a different position in the next year, 41 percent indicated the primary reason for the change was career advancement, up from 32 percent in 2012.

As with almost every preceding survey, respondents ranked “innovative leader” as the most powerful driver in choosing the best companies. This year, that was followed by “treats employees with respect,” “socially responsible,” “loyal employees,” “clear vision,” and “quality research” (see Driving Characteristics table, page 502).

Three companies, Biocon Limited (from #19 to #6), Eli Lilly and Company (from #17 in 2011 to #5), and Novartis (from #11 to #8) jumped up to the top 10 list, after having spent previous years in the second tier of the top 20 rankings. Newcomer AbbVie, a biopharma

company that spun off from Abbott Laboratories on January 1, came in at an impressive #4 (Abbott ranked #15 in 2012 and #18 this year). “We are a biopharmaceutical company, which our employees recognize combines leading-edge biotechnology with the expertise and long track record of a pharmaceutical leader,” says **Jim Sullivan**, vice president of pharmaceutical discovery for AbbVie in North Chicago.

Vertex Pharmaceuticals Incorporated (#3), Millennium: The Takeda Oncology Company (#7), Boehringer Ingelheim (#9), and Biogen Idec (#10) round out the top 10 employers (see chart above for full top 20 list).

Innovation Above All

Not surprisingly, scientists are happiest when drug development is driven forward by sound science and data, rather than the size of a potential drug’s market.

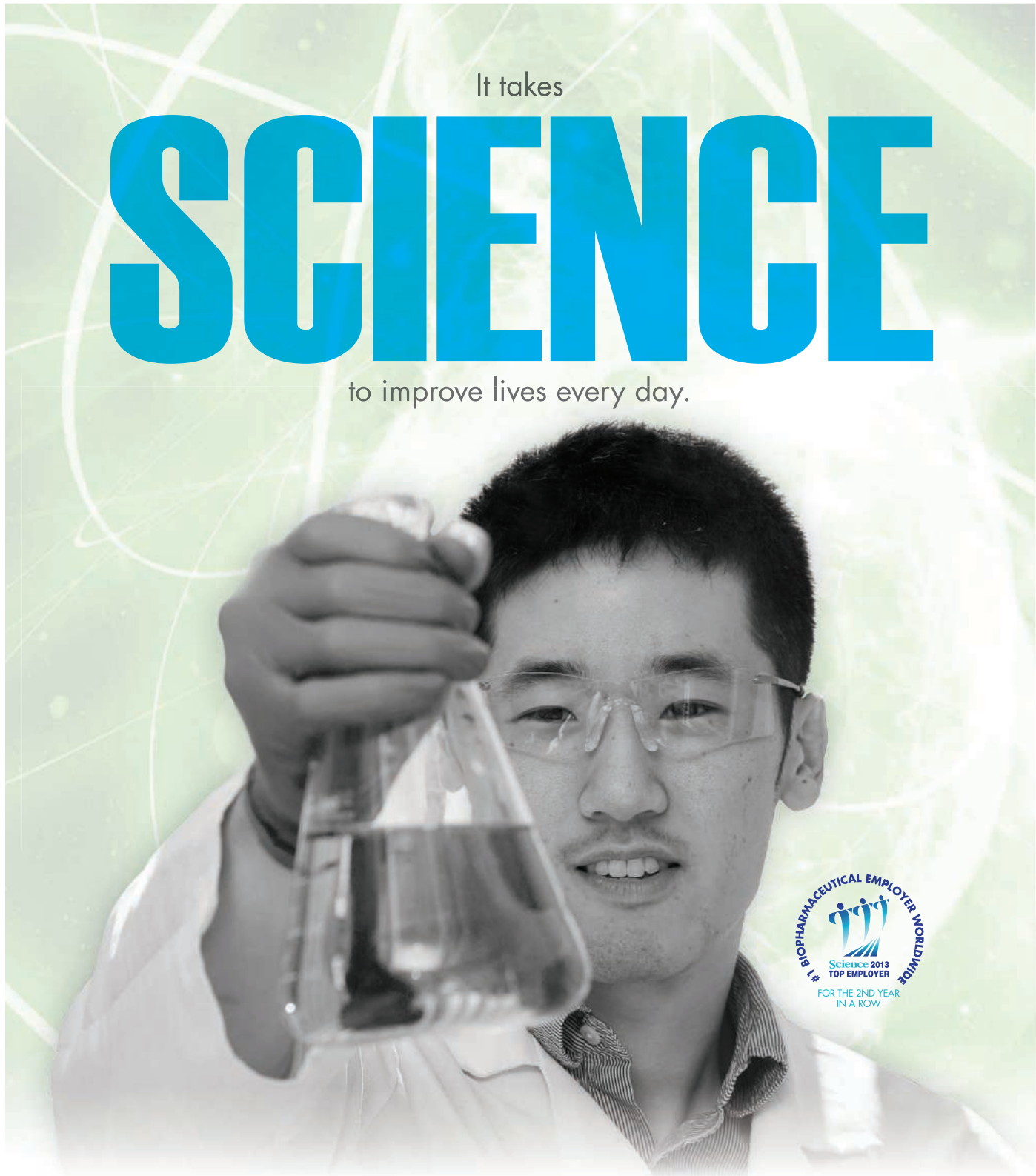
“We do not look at markets and sales,” says **Mark Fishman**, president of the Novartis Institutes for BioMedical Research (NIBR), the R&D branch of Novartis. “I actually forbid review of any of those parameters in any document that I receive from my sci-

continued>

It takes

SCIENCE

to improve lives every day.



The drive to improve people's lives. It's what turns great ideas into life-changing accomplishments. And it's what defines each and every member of Regeneron's talented and devoted team, universally committed to developing better medicines for serious illnesses. Here, we'll challenge you to expand your knowledge, enhance your expertise and achieve excellence—every day. And we'll support you with world-class resources, outstanding rewards and countless opportunities for professional development and advancement. So you can grow our company's capabilities, while achieving your most ambitious career goals.

Join us at the forefront of new discoveries.
careers.regeneron.com

REGENERON
science to medicine®

Survey Demographics

Gender:

57% Male, 38% Female, 5% No response

Experience:

65% have 10 or more years work experience

Highest Degree Earned:

41% Doctorate, 27% Master's, 27% Bachelor's, 5% Other

Company Type:

37% Pharma, 36% Biotech, 19% Biopharma, 3% University, 5% Other; More than 4 out of 5 work in private industry

Nature of Work:

25% Development, 25% Applied Research, 19% Basic Research, 10% Administration/Executive, 8% QA/QC/Regulatory Affairs, 14% Other (respondents were able to choose more than one response)

Geography:

81% from North America, 10% from Europe, 7% from Asia/Pacific Rim, 2% from rest of world



tists.” Instead, NIBR’s efforts are motivated by unmet medical needs, particularly in conditions with a well-understood disease mechanism. Often, these are rare diseases that NIBR pursues with the philosophy that a success in a well-understood molecular pathway will lead to discoveries that apply to more common ailments affected by the same pathway.

“If you have a culture dedicated to science, then you have a lot of individual discovery and freedom with a scientific critique, but not a business critique,” says Fishman, a cardiologist who has led NIBR’s 6,000 employees from its Cambridge, Massachusetts headquarters for the last decade. “We encourage people to ask important basic questions and important clinical questions, rather than focus on some tiny deliverable.”

The breadth of innovation in pharmaceutical research is expanding, with pushes in personalized, tailored medicine opening up new frontiers. Although drug development pipelines are still dominated by traditional small molecule inhibitors and monoclonal antibody biologics at clinical stages, earlier stages are witnessing the emergence of entirely new entities.

From oncology to infectious disease, it’s becoming clear that multiple drugs attacking multiple targets are necessary for true cures. But the high costs and complex delivery of biologic drugs severely limits how many can be given to a patient at once. So drug researchers are turning to new ways to combine therapies into single treatments.

“Genentech is making major advances and innovations in antibody engineering,” says Genentech’s Lee-Karlon. The firm of 1,200 scientists has been a pioneer in biologics since its founding in 1976, including blockbuster cancer drugs Avastin, Herceptin, and Rituxan. Current projects include a dual-action antibody in which both arms can block either cancer target, HER3 or EGFR. Perjeta is another antibody designed to block dimerization of the HER2 receptor in breast cancer and was approved by the U.S. Food and Drug Administration in 2012.

Lee-Karlon says the Perjeta project in particular took persistence on a level not often supported by other drug makers. “At Genentech, there is a willingness to dig deep and develop mastery [in a topic], which requires patience and iteration.”

Gilead Sciences has built a reputation as a leader in antiviral therapies, particularly with combination therapies for HIV and hepatitis C infections. In 2012, Gilead brought in almost \$8 billion in revenue from its HIV drugs including its three single-tablet regimens Atripla, Complera, and Stribild, approved in 2012 in the United States. The Foster City, California-based company’s willingness to out-do its own HIV therapies before they go off patent draws scientists in, says **Katie Watson**, senior vice president for human resources.

“We’re already innovating what could be the next generation drug after Stribild. I’m not sure others in our industry are always doing that.” With only 5,600 total employees and 2,400 in the R&D force, Watson says the company gives eight company-wide updates each year to ensure that all employees understand the science behind their products and know how research is progressing.

Bill Lee, senior vice president of research, says there’s a palpable excitement that a cure for hepatitis C virus (HCV) may be around the corner. Gilead’s new HCV drug sofosbuvir, expected to be approved by the end of the year, works in a combination therapy to supplant current therapy—a 6- or 12-months-long regimen of weekly interferon injections that cause flu-like symptoms.

“We’re on the verge of oral treatments that have the potential to eliminate the hepatitis C virus from the world,” says Lee. “For scientists here, every day they have the potential to change medicine. What you do in the lab can make a big difference.”

Sullivan of AbbVie, which has a competing HCV program, says the potential to have a remarkable, transformative impact on millions of patients’ lives motivates the company’s scientists. “We are starting to see data that suggests we are significantly increasing cure rates. That effort started with scientists doing an experiment in the lab close to 20 years ago trying to understand the virus better.”

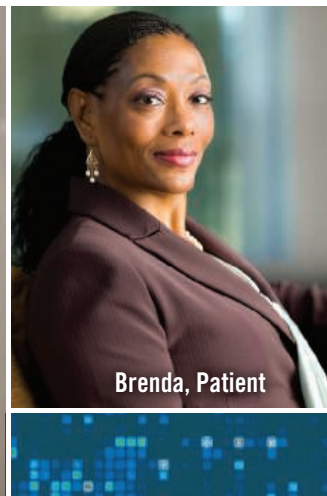
Hitting a Research Stride

At the #1 ranked top employer, Regeneron, the drive to bring new approaches to drug discovery—present since the company’s founding in 1988—has ripened into three marketed products, including the recently approved Eylea for wet, age-related macular degeneration and Zaltrap for metastatic colorectal cancer. Both are “decoy receptors” derived from the company’s Trap technology. Traps incorporate portions of human receptors to mop up overzealous signaling molecules in the body.

“[Our] overarching principle is to innovate around all the bottlenecks that occur in drug development,” says Stahl. “We have carefully examined that process to come up with ways to speed up the timeframes of all the pinch points.”

Technological platforms, such as the transgenic VelocImmune mouse, allow Regeneron to develop highly selective human monoclonal antibodies. As Stahl explains, the technology capitalizes on the ability of the mouse’s immune system to efficiently select, from among millions of antibodies generated, the ones that bind their target best, fold-up properly, persist in the blood, and have the best drug-like properties. And while other companies have mice that **continued>**

Life Inspired.



Passionate About Our Science and Our People

We're passionate and rigorous about our science. For more than 30 years, Genentech has been at the forefront of the biotechnology industry, using innovative science to develop breakthrough medicines that improve the lives of people with serious or life-threatening diseases. We're also passionate about our people, our most important asset.

We are currently seeking talented postdoctoral research fellows for the following areas:

Postdoctoral Research Fellow – Bioinformatics –
Req. #392630

Postdoctoral Research Fellow – Cancer Genomics –
Req. #412906

Postdoctoral Research Fellow – Discovery Oncology –
Req. #416470

Postdoctoral Research Fellow – Discovery Oncology –
Req. #417644

Postdoctoral Research Fellow – Dixit Lab –
Req. #417544

Postdoctoral Research Fellow – Early Discovery Biochemistry –
Req. #415690

Postdoctoral Research Fellow – Immunology –
Req. #417621

Postdoctoral Research Fellow – Immunology –
Chan Lab – Req. #417490

Postdoctoral Research Fellow – Metabolic Disease –
Req. #414715

Postdoctoral Research Fellow – Molecular Imaging –
Cancer Immunotherapy – Req. #417862

Postdoctoral Research Fellow – Neurodegeneration –
Req. #416129

Postdoctoral Research Fellow – Protein Engineering –
Req. #415005

Postdoctoral Research Fellow – Vascular Biology –
Req. #407281

Postdoctoral Research Fellow – Cancer Biology –
Req. #417778

Now a member of the Roche Group, Genentech has multiple medicines on the market for cancer and other serious illnesses. We are an equal opportunity employer and in 2013, we were named “top employer in the biopharmaceutical industry” by Science magazine for the 13th time.

Join us as we continue to tackle medicine's most challenging problems and live a life inspired. For complete position descriptions and to apply, please visit careers.gene.com and enter the Requisition number in the keyword search field.

Driving Characteristics of Top Employers

2013

1. Innovative leader in the industry

2. Treats employees with respect

3. Socially responsible

4. Loyal employees

5. Has a clear vision

6. Does important, quality research

2012

1. Innovative leader in the industry

2. Treats employees with respect

3. Socially responsible

4. Loyal employees

5. Does important, quality research

6. Makes changes needed

Driving characteristics are listed in descending order of impact on overall employer rankings
Shaded background indicates the characteristics in common for the two years.

generate human antibodies too, “our mice make more diverse antibodies.”

Stahl says the company was criticized early on for focusing too much on developing technologies instead of pushing a product to market quickly. “It took us awhile, but we are definitely hitting on all pistons now,” Stahl says. “All the investment we made in technology and research has put us in an enviable position—everything we’ve ever put into the clinic has been internally derived, and now we have more than we can possibly develop.”

Indeed, Regeneron’s formula appears to be paying off—every drug put into clinical trials in the last five years has been a monoclonal antibody from the VelocImmune mouse, including the LDL cholesterol-lowering drug that spent less than two years in preclinical development. Although the company has grown from 30 employees when Stahl started in 1991 to more than 2,100 today, it is still small enough that employees get the opportunity to work on multiple projects reaching the clinic—unlike many of their counterparts at larger companies.

Stahl says there’s a healthy sense of rigor among employees who don’t shy away from challenging each other’s data. “It’s a company that fosters creativity and tends to hire really smart people,” says **Venus Lai**, executive director, VelociGene operations and transgenic biology at Regeneron. “We may not always share the same opinions in the conference room, but we have in front of us one common goal.”

Regeneron has an “inverted pyramid” structure, says **Paul Davies**, vice president of human resources, which places the troops of scientists at the top, supported by everyone else within the organization because it recognizes that science creates the company’s value.

“That is a very different look and feel from the usual corporate structure,” Davies says. “We know our people are a real source of strategic advantage for us, so we try to create the kind of working environment where people are happy.”

Work Hard, Play Hard, Work Some More

In this year’s survey, 75 percent of respondents said they were not likely to seek a different job and at Regeneron turnover is a mere 4 percent. Making sure workers stay in their happy places means visits from the frozen yogurt truck on Thursdays, raffles for New York Yankees

and Mets baseball tickets, and plenty of celebrations.

“It sounds silly, but there’s always an abundance of food here. If there’s an opportunity to celebrate, then we do it and we don’t have to ask permission,” says Davies. Lai confirms, warning new employees of the “Regeneron 15”—pounds they might gain attending events like the annual Cheesy Hawaiian Shirt Day.

Nothing captures the work-hard-play-hard ethic at Genentech better than the “Geeknam Style” video the IT team put together to show potential hires how much fun it is to work there. In the parody of the Korean pop hit Gangnam Style, Andy Wang, principal enterprise archi-

tect, leads Genentech employees in the crazy-pony dance around the campus that hugs the west side of the San Francisco Bay. In California casual attire, employees strut and shimmy among the computer servers, around the conference room, and in the elevators.

“It really illustrates how free-spirited and fun it is here. We’re not afraid to make fun of ourselves, especially our leaders,” says **Elizabeth Majoch**, senior staffing manager at Genentech.

Genentech’s sabbatical program is a unique recognition of hard work in which, after six years of service, employees are granted six weeks of paid time off to recharge however they wish. When they return, colleagues decorate their office with reminders of their exotic travels or pursuits.

Stepping away from the intensity of research is also encouraged on Lilly’s Indianapolis headquarters campus, which boasts a full-service fitness center, outdoor soccer field and track, and the REVelI eatery, with a pub and patio open for after-hours drinks.

“We all believe in taking a break, getting some exercise, and then coming back to work—it stimulates better thinking,” says **Terri Grant**, vice president for human resources for Lilly Research Laboratories.

More Than a Little Respect

Supporting employees at these top organizations means treating them with respect—the second most important driver of the 2013 survey rankings—and trusting them to manage their own time, schedules, and family commitments.

At Biocon, which debuted at #19 last year and leapt to #6 this year, more than a quarter of their R&D workforce of 500 are women. **Ravi Dasgupta**, group head of human resources in Bangalore, says the company extends a level of support to working mothers that is uncommon in India. Women can extend their maternity leave well beyond the statutory three months with other kinds of leave or even unpaid time and be assured of a position when they return. Upon their return, many women work a three-quarter-time schedule and there is on-site daycare.

Biocon has been particularly successful at recruiting ex-pat Indians back from the United States, with 70 such employees hired in the last decade. **Abhijit Barve**, president of R&D and regula- **continued>**

A NEW COMPANY, A RICH HERITAGE

We are a global, research-based biopharmaceutical company that combines the focus and passion of a leading-edge biotech, with the expertise and results of a long-established pharmaceutical leader.

Redefining what is possible is our business, and our passion. We aim to help patients live healthier lives. Through new technologies and medicines, we harness breakthroughs and improve healthcare on a global basis.

Join our research and development efforts to make a meaningful difference for patients and their families.

Discover the possibilities with AbbVie. Apply your passion here.

www.abbviecareers.com



www.facebook.com/abbviecareers

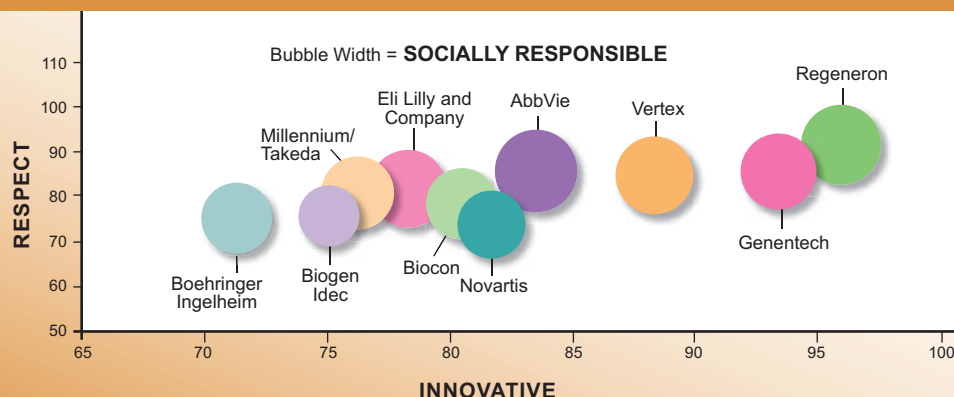


www.linkedin.com/company/abbvie

An equal opportunity employer, AbbVie welcomes and encourages diversity in our workforce.
Equal Opportunity Employer M/F/D/V



Comparison of Top Ten's Top Characteristics



Comparison of the top 10 companies on the basis of the top three drivers (scored out of 100): *Innovative leader in the industry* (x axis), *Treats employees with respect* (y axis), and *Socially responsible* (bubble width).

tory sciences, himself made the move in 2010 after almost 15 years in the United States. He was attracted by the opportunity to have more responsibility at a younger organization and by the entrepreneurial spirit of the company, embodied by its founder and chairwoman, Kiran Mazumdar-Shaw.

Biocon's open-door culture lets every employee feel comfortable sharing their views with the senior management. "They will always get a proper hearing. Anyone coming in will be respected," says Dasgupta.

Respect for individual ways of thinking makes it enjoyable to work at NIBR too, says **Elizabeth WIELLETTE**, an investigator in the Developmental and Molecular Pathways group. "People appreciate when you think as far outside the box as you can. It's better to suggest something crazy or on the edge of feasible and then bring it to something more realistic."

NIBR has also gone to great lengths to respect the needs of some of its least outspoken employees, the introverts. During recent overhauls of several NIBR campuses, Fishman, a self-described introvert, made certain that "little sheltered nooks and crannies" were added back to the buildings with open-lab space so that introverts would have spaces to work in where "they can't be seen and cannot see others."

Making Failure an Outdated Concept

Trusting people to make the right decisions is key, but so is the flip side of that, says **Michael Fralish**, a Regeneron staff neuroscientist. "There's not a lot of blame that happens here. If a project legitimately doesn't pan out, then let's move on. That's really unique."

Abolishing the concept of failure and celebrating the data for what it reveals, whether positive or negative, is part of the scientific culture spearheaded by **Jan Lundberg**, president of Lilly Research Laboratories.

After all, failure is a hallmark of innovation, he says. "You have to take risks and open the door to the unknown. Sometimes there's nothing behind the door and sometimes you find a golden nugget."

Just as Lundberg views failures as learning steps, he also views the coming pay freeze in 2014 for Lilly employees as a relative positive. The pay freeze is projected to save around \$400 million to offset the

loss of revenues from top-selling drugs Cymbalta and Evista that go off patent this year. "I see this as a difficult choice, but it's the right thing to do to preserve research capability for the future. We are saving jobs by doing this," says Lundberg.

Scientists on the Top Rung

Lilly's culture of celebrating data is strengthened by having a Ph.D. scientist, CEO **John Lechleiter**, leading the whole company. Lechleiter first joined the company within days of defending his thesis in 1979.

"I'm a Lilly lifer," says Lechleiter. "My approach has been to hire really outstanding scientific leaders, and to support their efforts to hire top-notch scientists." He says researchers are inspired by Lilly's his-

toric achievements in medicine, such as bringing insulin to patients with diabetes in the 1920s, and the company's willingness to make big bets in areas such as Alzheimer's disease. "It's what they signed up to do, and we're enabling them to do it."

As a Ph.D.-holding CEO, Lechleiter is almost unique among the largest pharmaceutical firms, but having scientifically trained top executives is a common theme at many of the high-ranking organizations interviewed here. Gilead's CEO, COO, and CSO are all Ph.D. scientists who have been with Gilead for 23 years out of its 26-year history.

When scientist leaders guide companies for decades, it also ensures that a science-based culture remains in place even through major transitions or rapid expansions, says Stahl of Regeneron. The company's founders, CEO Leonard Schleifer and CSO George Yancopoulos, both M.D./Ph.D.s, check in with their scientists at the bench. "We are ruthless about rigor and challenge," because it results in better products, says Stahl. "The most senior people still participate in that process every single day."

Companies like Regeneron that place science at the center of operations come out ahead. Hard, challenging work is expected, but also rewarded and recognized—then balanced by activities like kayaking in Tarrytown's nearby Hudson River, volunteering with tuberculosis education in South Africa (Lilly), or literally dancing in the Bay Area streets (Genentech). Most of all, these companies appreciate individual ways of thinking and pushing the boundaries of medical research.

"We have demands on our people. You are supposed to deliver," says Lilly's Lundberg. "But my colleagues have always had a lot of freedom to deliver. Innovation is done by unique individuals." The best ideas rise to the top, he says, when you let workers run like ants "in all different directions, but with the same goal."

He thinks competitive scientists get bored if they aren't stressed. But, he says, the high expectations for drug development should be met "by having respect for people, with integrity and with excellence. That's how you win in the long-term."

Kendall Powell is a freelance science writer based in Lafayette, Colorado.

DOI: 10.1126/science.opms.r1300137



What will YOU discover in Switzerland?

Hailing from across the globe, six scientists tell why they brought their talent and curiosity to Switzerland and Roche pRED.

Katharina Kreymborg, a scientist in Human Immunology, studied in Germany and Zurich and spent the last three years in New York as a postdoc:

"I joined Roche pRED to be part of a global team that works together towards one goal of value. I'm excited about the mix of basic research and scientific curiosity and the goal-oriented way of working."



Molecular modelling at Roche

Earning her PhD in Quebec, Canada, **Annie Moisan** did her postdoc at Harvard. Now a scientist in Cardiovascular and Metabolism Discovery, Basel, she says:

"Switzerland shines as a world-leading science nation. Talented and motivated scientists from across the globe bring diversity and share a common will to innovate. The quality of life is outstanding and travel opportunities are endless."

Gabriela Burian, Ophthalmology Early Program Leader, Basel, studied medicine in both Romania and Atlanta, Georgia.

"I came to Roche pRED for the unique opportunity to apply my clinical research background in the early research and development setting and expand my learning in the area of scientific discovery with an extra-ordinary group of scientists."

Oliv Eidam, a scientist in the Cheminformatics & Statistics group, Basel, studied in San Francisco and Zurich: "If you want to work here, contacts are everything. Get to know pRED people at conferences, make an internship or invite yourself to present your work in a seminar – that's what I did."

A native of Italy, **Luca Ferrari**, Small Molecules Bioanalytics, Non-Clinical Safety, says:

"I joined Roche pRED because of its well-known reputation within the scientific community. pRED scientists possess high levels of education and experience, and I also like the diversity/multicultural environment. Roche pRED is a great place to work!"

"I work together with the best scientists, create innovative ideas and translate these into the clinic," explains **Roger Suttmuller**, Group Leader Infection Immunology in Schlieren, who most recently lived in Belgium and previously worked in The Netherlands.

"If you like an open atmosphere, teamwork, and want to put science to work helping patients – what are you waiting for? Apply!"

www.careers.roche.com



Our Roche scientists from left to right: Roger Suttmuller, Oliv Eidam, Annie Moisan, Luca Ferrari, Katharina Kreymborg, Gabriela Burian

biogen idec

Caring Deeply.
Changing Lives.

LIFE-CHANGING WORK *More breakthroughs, fewer barriers*

It's why you pursued a career in life sciences: an opportunity to change lives – maybe even your own.

Biogen Idec is seeking fearless, creative, entrepreneurial scientists across a variety of disciplines to help create the next generation of innovative drugs for patients and unmet medical needs.

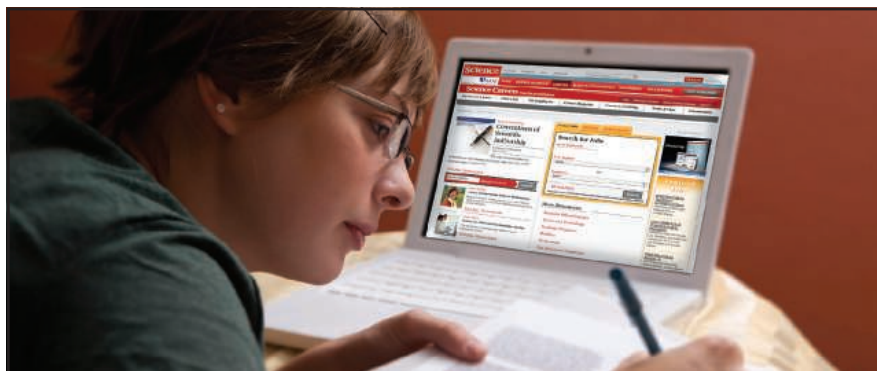
You'll use the latest translational and computational techniques to solve clinical problems and discover new biology. You'll do great science alongside remarkable people. It's life-changing work – and it's waiting for you at Biogen Idec.

biogenidec.com/careers

Biogen Idec has immediate openings in the following areas:

- Molecular Biology
- Immunology
- Fibrosis
- Neurobiology
- Pathology
- Cell Biology
- Antibody Engineering
- Computational Biology
- Postdoctoral Program

Biogen Idec is an Equal Opportunity/Affirmative Action Employer M/F/D/V.



AAAS is here – helping scientists achieve career success.

Every month, over 400,000 students and scientists visit ScienceCareers.org in search of the information, advice, and opportunities they need to take the next step in their careers.

A complete career resource, free to the public, *Science Careers* offers a suite of tools and services developed specifically for scientists. With hundreds of career development articles, webinars and downloadable booklets filled with practical advice, a community forum providing answers to career questions, and thousands of job listings in academia, government, and industry, *Science Careers* has helped countless individuals prepare themselves for successful careers.

As a AAAS member, your dues help AAAS make this service freely available to the scientific community. If you're not a member, join us. Together we can make a difference.

To learn more, visit
aaas.org/plusyou/sciencecareers



For more than 130 years, Lilly has been dedicated to meeting the health care needs of people in the United States and around the world. We address these needs primarily by developing innovative medicines—investing a higher percentage of our sales in research and development than any other major pharmaceutical company. If you are interested in being considered for employment with a “Best in Class” Pharmaceutical company, please review the following opportunity:

Job I.D. Number 6643 - Lilly seeks a Director/Senior Medical Advisor for its Autoimmune Product Development area to lead global phase 2/phase 3 development programs in indications, including RA, psoriasis, and diabetic nephropathy. This position also serves as the clinical leader for a compassionate use program in rare childhood multisystem inflammatory diseases, including CANDLE syndrome. Specific duties include: setting strategy, trial design and development, approval of protocols, investigator/opinion leader interaction including advisory boards and global health authorities, overseeing conduct of studies, data analysis, and scientific communications, and managing regulatory submission planning and execution. This position requires an MD or similar medical degree and experience in autoimmune research. Salary: \$250,008 per year.

Please submit resume to www.lilly.com/careers and cite the relevant job title and job I.D. number in your submission.

Lilly is an Equal Opportunity Employer that values the strength diversity brings to the workplace.

Lilly

A close-up photograph of several microfluidic chips, each with a small blue droplet at its tip, set against a blurred background of more chips.

ENGINEERING AT ILLINOIS

DRIVE YOUR VISION

ENDOWED CHAIRS AND PROFESSORSHIPS IN BIOENGINEERING

Bioengineering is revolutionizing 21st century healthcare worldwide. But to have the greatest impact, the best minds have to work together across a variety of fields. At the University of Illinois, that interdisciplinary attitude and the desire to deliver safe, effective, affordable medical technologies drive us. They've led to breakaway work in imaging, biosensing, cellular mechanics, and biophysics. Now we're expanding our team. Thanks to the \$100 million Grainger Engineering Breakthroughs Initiative, we're creating more than 35 new endowed professorships and chairs in Bioengineering and other fields. If you're ready to drive the future of Bioengineering, Illinois is the place for you.

GraingerInitiative.engineering.illinois.edu



Illinois is an Affirmative Action/Equal Opportunity Employer. www.inclusiveillinois.illinois.edu.
Full consideration will be given to applications and nominations received by December 16, 2013.

The Faculty of Mathematics and Natural Sciences at the University of Cologne invites applications for the position of a

Full Professorship (W3) for Experimental Physics Condensed Matter Physics (Physics Institute II)

Candidates should have an outstanding research record in experimental condensed matter physics that expands and complements the ongoing research activities at the Department of Physics at the University of Cologne. He/She should play a leading role in condensed matter research at the University of Cologne. Possible research areas include topological matter, interfaces and/or thin films, control of quantum matter, or strongly correlated electron systems. The new professor is expected to teach physics both at the undergraduate and the graduate level, and to be actively involved in the Bonn-Cologne Graduate School of Physics and Astronomy.

The professorship will be integrated into the University's Center of Excellence on "Quantum Matter and Materials" (QM²). QM² is a focused strategic alliance between theoretical physics, experimental physics, chemistry, and mathematics and integrates scientists and research institutions in the Cologne area. It is strongly supported by the University's master plan and by the Excellence Initiative of the German federal and state governments.

Qualification requirements are in accord with the North Rhine-Westphalia University Law and include an excellent track record in research and teaching. Women are strongly encouraged to apply.

The University of Cologne is an equal opportunity employer in compliance with the German disability laws. Applications should include a letter of motivation with a teaching and research statement and the usual documents (CV, complete publication list highlighting the 5 most important papers, information on external funding, academic achievements and honors, and 3 references) as well as a completed form provided at <http://www.mathnat.uni-koeln.de/mnfapplication.html>.

Applications should be submitted preferably via e-mail no later than **December 1, 2013** to: **Professor Dr. Karl Schneider, Dean of the Faculty of Mathematics and Natural Sciences, University of Cologne, Albertus-Magnus-Platz, 50923 Cologne, Germany, e-mail: mnf-berufungen@uni-koeln.de**

www.uni-koeln.de

Universität
zu Köln



Biology of Parasitism Assistant Professor

The Department of Cellular Biology at the University of Georgia invites applications for a full-time tenure track Assistant Professor position to begin August 2014. We seek an individual who has a research program in cellular and molecular parasite biology, with particular interest in systems biology approaches. The Department has strong representation in the Center for Tropical and Emerging Global Diseases, one of the world's leading centers for parasite research. The position includes a very competitive salary, excellent laboratory space and a generous start-up package.

Applicants must hold a PhD degree and have at least two years postdoctoral training. The successful candidate will have teaching responsibilities in our undergraduate and graduate programs.

Applicants should submit a cover letter, curriculum vitae, research statement and teaching philosophy at: <https://secure.interfolio.com/apply/23064>, and request submission of three confidential letters of recommendation via Interfolio. Review of applications will begin on **November 15, 2013** and continue until the position has been filled. Contact cbsearch@uga.edu with questions.

The Franklin College of Arts and Sciences and the University of Georgia is committed to increasing the diversity of its faculty and students, and sustaining a work and learning environment that is inclusive. Women, minorities and people with disabilities are encouraged to apply. The University of Georgia is an Affirmative Action/Equal Opportunity Institution.



SCIENTIFIC PROGRAM LEADER Associate/Full Professor (tenure eligible) Position Number: F35670

Hire Date: January 1, 2014

Deadline: Open until Filled

The Massey Cancer Center of Virginia Commonwealth University (VCU), an NCI-designated Cancer Center and VCU School of Medicine are recruiting a senior scientist to lead its Radiation Biology and Oncology (RBO) Scientific Program. One of five scientific programs with the Massey Cancer Center, and one of only nine programs in NCI-designated Cancer Centers in the country with an emphasis on radiation sciences, the RBO has a scientific focus in the areas of DNA repair, tumor microenvironment, and the impact of inflammation on tumor radio responsiveness and normal tissue radio sensitivity. The RBO program also is one of the only two programs in the Cancer Center with an active clinical trial component. A multidisciplinary program with sixteen members representing four different departments and two schools, membership in the RBO consists of five laboratory scientists, three medical physicists, one biostatistician and seven clinicians.

This is a tenure-track appointment at the rank of Associate Professor or Professor in the VCU School of Medicine, commensurate with the candidate's experience. The successful candidate will have a track record of sustained extramural funding, publication and recognition as a leader in his/her field of study. He/she will be expected to provide scientific direction, foster inter- and intra-programmatic collaborations leading to growth of the program, and expand the program's translational research component. He/she must have a Ph.D. and/or M.D. in a relevant scientific area. The successful candidate must have demonstrated experience working in and fostering a diverse faculty, staff, and student environment or commitment to do so as a faculty member at VCU. Richmond is a mid-sized metropolitan area with close proximity to Washington, D.C., the beautiful Blue Ridge Mountains, and scenic coastal areas.

For further details, please contact: Donna Berrier; Phone: **804-628-1322**; email: dberrier@vcu.edu. Applications shall be forwarded to: **Donna Berrier, CAO, VCU Massey Cancer Center, 401 College Street, P.O. Box 980037, Richmond, VA 23298-5083** or email at dberrier@vcu.edu.

Virginia Commonwealth University is an equal opportunity/affirmative action employer. Women, minorities, and persons with disabilities are encouraged to apply.



南京大學
NANJING UNIVERSITY

Nanjing, CHINA

Founded in 1902, Nanjing University is one of the oldest and most prestigious institutions of higher learning in China. As a key comprehensive university with an array of outstanding faculty members, it has enjoyed coordinated development in humanities, social sciences, natural sciences, technological sciences, life sciences, modern engineering and management and so on. With the motto of "Sincerity with Aspiration, Perseverance and Integrity," Nanjing University carries the spirit of constant striving for educational and academic excellence. Today's Nanjing University invites outstanding scholars of all nationalities to join us in the mission to build this university into a world-class comprehensive research university with a global vision.

Position: Distinguished Professor

Offered by Thousand Talents Program/ Chang Jiang Scholars Program/ Deng Feng Scholar Program A

- The applicant should hold a professorship/associate professorship (or an equivalent position) in a prominent overseas university (or research institutes)
- The applicant demonstrates outstanding capabilities in scientific innovation, whose research capabilities and achievements are recognized by peers as at leading level.

Position: Young Talents Professor

Offered by Thousand Young Talents Program/Deng Feng Scholar Program B

- The applicant should hold an assistant professorship (or an equivalent position)/research fellowship in a prominent overseas university (or research institutes)
- The applicant should have been a top talent among peers and shown a strong potential to be a future leader in his/her area.

The position offers adequate scientific resources, abundant research funding, attractive salary and generous reallocation package, including the opportunity to buy an apartment in an inter price rate and subsidy.

Interested candidates please visit <http://rczp.nju.edu.cn/>

Florida State University

Strategic Faculty Recruitment in Energy and Materials



Florida State University is continuing its *major interdisciplinary initiative in the areas of Energy and Materials*. During the 2013-14 academic year the University will be recruiting as many as nine tenure-track/tenured faculty members to supplement the three faculty hired last year in these areas. This search is open with respect to rank and academic department. Successful candidates are expected to have a synergistic impact on existing research programs in the University's departments and interdisciplinary centers as well as develop new areas. Sustained pursuit and growth of collaborative, externally-funded research programs is an explicit goal.

We invite applications from researchers active in the broadly-defined area of materials science and materials engineering with an emphasis on, but not restricted to, materials for energy production, conversion, storage and utilization. Target research areas in this search encompass theory, computation, synthesis including molecular, macromolecular and inorganic, thin films and crystals, biomaterials, fundamental characterization, materials measurement, device construction and proof of concept testing and prototyping. Successful candidates will be offered highly competitive salaries and start-up packages, state-of-the-art research space and access to world-class instrumentation, computing and facilities in academic and interdisciplinary units.

Related strengths at Florida State University include programs in Biological Science, Chemistry and Biochemistry, Physics, and Scientific Computing in the College of Arts and Sciences, and in Chemical and Biomedical, Electrical and Computer, Industrial and Manufacturing and Mechanical Engineering in the College of Engineering. Complementing these programs are interactive centers including the National High Magnetic Field Laboratory, the Applied Superconductivity Center, the High Performance Materials Institute, the Aero-Propulsion, Mechatronics and Energy Center, and the Center for Advanced Power Systems. Linking these colleges and centers is a new Ph.D. program in Materials Science and Engineering complementing robust department-based doctoral programs in materials and related areas.

Florida State University is classified as a very high research activity, doctorate-granting institution with a student population approaching 42,000. In recent years, the University has made considerable investments in research infrastructure in the sciences and engineering disciplines. The University is located in Tallahassee, the capital of Florida, where residents have access to a broad range of cultural amenities afforded by the presence of three institutions of higher learning. The region boasts an abundance of springs, lakes and rivers as well as pristine beaches on the Gulf of Mexico.

Applicants are asked to provide a *single document* in pdf format containing a letter of application including the names and contact information of three professional references, curriculum vitae, and a two page narrative describing their research interests that should include a clear statement as to how the candidate would complement this inter-college effort at Florida State University. Applications must be sent electronically to materials2013.search@fsu.edu. Review of applications will begin on **November 1, 2013**. Additional information about the related programs at FSU and this faculty search can be obtained at http://www.research.fsu.edu/materials_search/.

Florida State University is committed to the diversity of its faculty, staff, and students, and to sustaining a work and learning environment that is inclusive. Women, minorities, and people with disabilities are strongly encouraged to apply. FSU is an Equal Opportunity/Access/Affirmative Action Employer.



ASSOCIATE/FULL PROFESSOR, CANCER MOLECULAR EPIDEMIOLOGY

(tenure eligible)

Position Number: F35680

Hire Date: January 1, 2014,

Deadline: Open until Filled

Position Summary: Virginia Commonwealth University (VCU) Massey Cancer Center, and the School of Medicine are jointly seeking outstanding candidates for a tenure-track associate professor/professor faculty position in genetic/epigenetic epidemiology of cancer. This position will include an Endowed Professorship and will support Massey Cancer Center's strategic plan to further develop research in cancer screening, prevention and control. NCI designation means that we play an important role in the leadership and shaping of the nation's cancer research efforts. Like NCI, our research mission is to eliminate the suffering and death caused by cancer.

VCU Massey Cancer Center earned National Cancer Institute designation in 1974 based on our world-class research programs, and we have retained that status ever since. Our researchers and physician-scientists conduct laboratory and clinical and prevention and control research to discover better ways to prevent, diagnose and treat cancer. Nearly 130 of these researchers are members of our preeminent research membership program, which is for those whose primary academic interests relate to cancer. Cancer Center research members hold faculty appointments across 22 academic departments, five schools and one college at Virginia Commonwealth University.

Richmond is a mid-sized metropolitan area with close proximity to Washington, D.C., the beautiful Blue Ridge Mountains, and scenic coastal areas.

The qualified candidate will have a medical and/or doctoral degree with published experience working with large data sets, expertise in the molecular epidemiology of cancer, demonstrated success in obtaining peer-reviewed funding for research projects, and experience in teaching molecular or classical epidemiologic methods. Experience in population-based studies of solid tumor cancers, including the use of tumor biomarkers and evaluation of recurrence risk, is strongly desired.

Candidates whose cancer research focus includes epigenetics, genetics, biomarkers, molecular epidemiology, environmental exposures or disease mechanisms in human populations, and applicants with strong methods/quantitative skills, are particularly encouraged to apply.

Educational Requirements: PhD and/or MD in relevant scientific area.

Qualification and Experience: Candidates for the rank of Associate Professor or Professor must have a strong record of scholarship with experience as a principal investigator and collaborator on funded epigenetic or genetic epidemiology research. Commitment to research translation into opportunities for prevention are viewed as strengths. Candidates must have demonstrated experience working in and fostering a diverse faculty, staff, and student environment or commitment to do so as a faculty member at VCU.

Application Process: Interested candidates should provide by e-mail: a cover letter, curriculum vitae, a brief description of research interests and future research directions, and contact information for three-to-four references, preferably as a single PDF file to: NAME, email, etc. of recruiter. Email Donna Berrier at dberrier@vcu.edu. Salary is commensurate with experience. Review of applications will begin immediately and continue until the position is filled.

For Additional Information: Donna Berrier, Phone: 804-628-1322, Fax: 804-828-5083, Web: <http://www.Massey.vcu.edu>

Virginia Commonwealth University is an equal opportunity/affirmative action employer. Women, minorities, and persons with disabilities are encouraged to apply.



THE HONG KONG UNIVERSITY OF SCIENCE AND TECHNOLOGY

Division of Life Science Faculty Positions

The Division of Life Science at The Hong Kong University of Science and Technology seeks applicants for multiple tenure-track positions at the rank of Assistant Professor. Candidates pursuing innovative research in areas including, but not limited to, aging and immunology are encouraged to apply. Applicants should have a doctoral degree and postdoctoral experience. They will be expected to establish an independent, internationally recognized research program and to contribute to the missions of the Division on undergraduate and graduate education.

The Division of Life Science (life-sci.ust.hk) currently has 35 faculty members from international background working on diverse areas of biological sciences. The University is located at a spectacular seaside setting just a short distance from downtown Hong Kong. Teaching and research are carried out in an outstanding intellectual environment rich in state-of-the-art infrastructure. The medium of instruction is English. The University is committed to increasing the diversity of its faculty and has a range of family-friendly policies in place.

Starting salary will be commensurate with qualifications and experience. Medical/dental benefits and annual leave will be provided. Housing benefits will also be available where applicable. Application materials including a cover letter, curriculum vitae, statements of a program of research and teaching interests, and contact information of three referees should be submitted to the Chair of Life Science Search and Appointments Committee (lifssearch@ust.hk). Review of applications will start in November 2013 and will continue until early 2014.

(Information provided by applicants will be used for recruitment and other employment-related purposes.)



ILLINOIS
UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

Genomics and Bioinformatics

The School of Integrative Biology at the University of Illinois at Urbana-Champaign invites applications for a full-time, nine-month, tenure-track assistant professor position in genomics and bioinformatics of eukaryotes. We are particularly interested in candidates with strong research programs in comparative genomics, population genomics, or gene networks. Candidates must have a PhD or equivalent in a relevant field, and postdoctoral experience is desirable. The successful candidate will be expected to develop an externally-funded research program, teach at undergraduate and graduate levels, and collaborate with other faculty both within SIB and elsewhere on campus to develop research initiatives in genomics and bioinformatics. The candidate will be housed in one or more of the departments of Animal Biology, Plant Biology, or Entomology. Target start date is August 16, 2014. Salary will be commensurate with experience.

The University of Illinois at Urbana-Champaign is a public land-grant university with more than 40,000 students and provides a highly collaborative and supportive academic environment. Opportunities for interactions exist with genomicists and bioinformaticians across campus including at the Institute for Genomic Biology, the National Center for Supercomputing Applications, and the Departments of Computer Science and Statistics, as well as our Masters in Bioinformatics graduate program. Support facilities include the KECK Center for Comparative and Functional Genomics and the High-Performance Biological Computing Center in the Roy Carver Biotechnology Center (<http://www.biotech.uiuc.edu/>).

To ensure full consideration, please create your candidate profile through <http://go.illinois.edu/GenomicsBioinformatics> and upload your application letter, curriculum vitae, summary of research and plans, teaching philosophy and experience, and contact information (including e-mail addresses) for three professional references by **November 29, 2013**. After a review of the candidate's record, the search committee may then contact the applicant about soliciting letters of reference. Applicants may be interviewed before the closing date; however, no hiring decision will be made until after that date. For further information contact Genomics and Bioinformatics Search Chair, sib@life.illinois.edu.

Illinois is an Affirmative Action /Equal Opportunity Employer and welcomes individuals with diverse backgrounds, experiences, and ideas who embrace and value diversity and inclusivity. (www.inclusiveillinois.illinois.edu).

**The central regulation of food intake and reward;
Molecular mechanisms and clinical pathology
such as obesity and anorexia**



UPPSALA
UNIVERSITET

Uppsala University, Department of Neuroscience
Unit of Functional Pharmacology

Post doctoral fellow

The research focuses on central regulation of food intake and reward mechanisms from both molecular and clinical perspective. We aim to understand the molecular mechanisms of obesity, anorexia and addiction using molecular biology orientated behavioural/pharmacological methods. We are interested in candidates with strong background in at least one of the following fields; transgenic mouse work, neuroanatomy, human genetics/epigenetics, human clinical studies or drosophila genetics. Our interest is restricted to post doctoral fellows with 1) PhD from Europe, America or Japan, 2) PhD degree within three years, 3) a record of published papers in highly rated international journals (at least three first name papers). The unit is led by professor Helgi B. Schiöth and includes one associate professor, 6 post doctoral fellows, 15 PhD students, guest researchers, short time students totalling more than 30 persons. We work actively to provide excellent individual career development with well document track record. Several of our previous PhD students and post doctoral fellows have become independent researchers. The post doctoral fellow will have opportunity to guide PhD students and there will be excellent possibilities to participate in integrated collaboration studies including the Uppsala University Hospital, Rudbeck Laboratory and Karolinska Institutet. Applications and enquires should be addressed to helgis@bmc.uu.se at any time, but not later than by **end of February 2014**. www.uu.se



**CHAIR, DEPARTMENT OF
PHARMACOLOGY AND TOXICOLOGY
COLLEGE OF PHARMACY and
SCHOOL OF MEDICINE**

UNIVERSITY OF UTAH HEALTH SCIENCES CENTER

The Department of Pharmacology and Toxicology (<http://www.pharmacy.utah.edu/pharmtox>) in the College of Pharmacy at the University of Utah is seeking a productive, collaborative, and innovative individual for the position of Department Chair. The successful candidate will also be appointed as Professor of Pharmacology and Toxicology. Applicants should have a PhD or MD degree, a strong track record of extramurally funded research and peer-reviewed scholarly productivity, a commitment to graduate training in pharmacology and toxicology, and an understanding of current issues in the teaching of pharmacology to professional medical and pharmacy students. The successful candidate should have excellent leadership and interpersonal skills, be a team player and demonstrate a commitment to working with and mentoring a diverse group of faculty and trainees.

Applicants must apply online at: <http://utah.peopleadmin.com/postings/26824>. For further information, please contact **Dr. Darrell Davis**, Chair of the Search Committee at darrell.davis@pharm.utah.edu

The University of Utah is an Equal Opportunity/Affirmative Action Employer and educator. Minorities, women, and persons with disabilities are strongly encouraged to apply. Veterans preference. Reasonable accommodations provided. For additional information: <http://www.regulations.utah.edu/humanResources/5-106.html>

The University of Utah values candidates who have experience working in settings with students from diverse backgrounds and possess a strong commitment to improving access to higher education for historically underrepresented students.

The University of Utah Health Sciences Center is a patient focused center distinguished by collaboration, excellence, leadership and respect. The University of Utah Health Sciences Center values candidates who are committed to fostering and furthering the culture of compassion, collaboration, innovation, accountability, diversity, integrity, quality and trust that is integral to the mission of the University of Utah Health Sciences Center.



UNIVERSITY OF
TORONTO



Donnelly Centre
for Cellular + Biomolecular Research

Assistant Professor - Integrative Biology
Faculty of Medicine
The Donnelly Centre
Deadline: December 1, 2013 or until filled

The Donnelly Centre at the University of Toronto invites applications to fill one Principal Investigator position. Appointments will be at the rank of Assistant Professor, in the tenure-stream. The appointment will begin on July 1, 2014 or on a mutually agreeable date.

We seek a colleague whose research program and interests will strengthen our existing scientific community, which encourages interdisciplinary collaboration. "Specific areas of interest include: chemical genomics and biotherapeutics (high throughput screening of compounds and other bioactive reagents for biological analysis), high throughput cell biology (genome-scale screens in mammalian or model organisms), enabling technologies (imaging of cellular processes, automation); systems biology (broadly defined); modeling of dynamic biological processes; analysis of gene regulatory networks; metabolomics; functional genomics."

Candidates must have a Ph.D. degree or equivalent, postdoctoral experience, and an established record of research accomplishment in a cognate scientific discipline. The successful candidate will be expected to mount an original, competitive, and independently funded research program, and to have a commitment to undergraduate and graduate education in a relevant academic department at the University of Toronto. Exceptional candidates with more seniority will also be considered. Salary will be competitive and commensurate with qualifications and experience.

The Donnelly Centre is a unique interdisciplinary research centre at the University of Toronto (<http://thedonnellycentre.utoronto.ca>) with a primary research mandate. The Donnelly aims to stimulate collaborative interactions at the interface of biology, chemistry, engineering and computer science in order to develop and apply new technologies for approaching the most challenging biological problems in the post-genomic era. The Donnelly currently houses 36 members from the faculties of Medicine, Pharmacy, Applied Sciences & Engineering and Arts & Science (Departments of Computer Science, Chemistry, Physics). Located in the heart of Toronto's research district, which is one of the largest and most active biomedical research communities in North America, the Donnelly makes use of open concept laboratory space to foster unconventional interactions among disciplines.

Letters of application should include a statement of current and long-term research interests together with a curriculum vitae and should be sent in confidence as a single PDF to:

sylvie.besnard@utoronto.ca / for
Professor Brenda Andrews
Chair, Search Committee
The Donnelly Centre, University of Toronto
Room 230, 160 College Street
Toronto, Ontario, Canada
M5S 3E1

Applicants should also arrange for three letters of reference to be sent electronically to the same email address. Applicants and referee letters will be accepted until **December 1, 2013**, or until the position is filled.

The University of Toronto is strongly committed to diversity within its community and especially welcomes applications from visible minority group members, women, Aboriginal persons, persons with disabilities, members of sexual minority groups, and others who may contribute to further diversification of ideas.

All candidates are encouraged to apply and will be considered; however, qualified Canadians and permanent residents will be given priority.



深圳大学

2013 Shenzhen University Oversea Recruitment

Shenzhen University (SZU) was founded as a public university in 1983 with the accreditation of the State Council of the People's Republic of China.

There are many famous scholars in SZU. Currently, there are 1,500 teachers on campus and about 60% of them have gotten Phd. Now, SZU has 2 Academicians of Chinese Academy of Sciences, 3 Academicians of Chinese Academy of Engineering, 2 members of "1000-Talents Scheme", 6 scholars who have won awards from the National Science Foundation for Distinguished Young Scholars, 4 scholars who have won awards from the "Chang Jiang Scholars Program", 3 members of New Century Millions of Talents Project at national level and 3 chief scientists in the National 973 Academic Program.

SZU, thirsty for talents, warmly welcomes numerous outstanding elites to join us as distinguished professors, associated professors or lecturers.

A. Distinguished professor

1) Eligibility: Candidates of the national "1000-Talents Scheme", "1000-Young-Talents Scheme", "Outstanding Youth", "Chang Jiang Scholars Program" and "100-Talents Scheme" of the CAS, professors or associated professors from overseas famous universities and outstanding scholars having fundamental academic influence.

2) Remuneration: It is yearly payroll for a distinguished professor, about RMB500,000-1,200,000. SZU will provide support in scientific research expenses and laboratory construction fee for a distinguished professor as well as in constructing his academic research team. Especially, Shenzhen local government will give scientific research expenses of RMB2,000,000 – 5,000,000 to a candidate who study such subjects as science, engineering and medicine and are eligible for the Peacock Program or Shenzhen High-Level Talent Program.

B. Professor, associated professor, lecturer and Liyuan Scholar Plan

(1) Professor, associated professor and lecturer

SZU warmly welcome overseas scholars who have achieved a Phd degree or have experiences of post-doctoral research and are competent for our positions of professor, associated professor and lecturer. Excellent candidates will be engaged as professors or associated professors directly by SZU according to their academic achievements.

Remuneration: the minimum annual salary is RMB310,000 for a professor, RMB250,000 for an associated professor and RMB180,000 for a lecturer.

Any professor, associated professor and lecturer could apply for Liyuan Scholar Plan, Peacock Program or Shenzhen High-Level Talents Program. If any person achieved any of the above plans, he could apply to Shenzhen Government for different subsidies by relevant plan.

(2) Liyuan Scholar Plan

Any teacher could apply for this plan. There are three levels for this plan, such as "Liyuan Leading Scholar", "Liyuan Outstanding Scholar" and "Liyuan Excellent Youth" respectively.

1) Three-level scholars: Liyuan leading scholar will be awarded to full-time teachers as a top-leader in his academic area. Liyuan outstanding scholar will be awarded to full-time teachers, aged under 45 and having 3 years' working experiences. Liyuan excellent youth will be awarded to young scholars aged under 35 who had gotten Phd and had one year's academic research experience in relevant research institutions.

2) Award standards: SZU will provide a living allowance for Liyuan leading scholars in 5 years, including two levels, RMB500,000 per year At the first level and RMB150,000 per year at the second. SZU will give a living allowance to Liyuan outstanding scholars in 3 years, RMB100,000 per year. SZU will give a living allowance to Liyuan excellent youth in 9 years at the most, RMB100,000 per year.

C. Shenzhen Oversea High Level Talents Policy

1) Candidates: overseas experts or overseas scholars. There are three levels for this plan, Level A, B and C respectively

2) Remuneration: candidates will receive an award of RMB500,000-1,500,000. Candidates whose research program is in such subjects as science, engineering and medicine respectively could enjoy scientific research expenses from RMB200,000 to 5,000,000. Talents at Level A could apply for scientific research expenses of more than RMB5,000,000.

Contact Us

For more information, please visit <http://www.szu.edu.cn>. If you're interested, please send your CV and relevant materials to any of the following email addresses:

Miss Liyun liyun@szu.edu.cn, 0086-755-26536111
Miss Gaoying gaoying@szu.edu.cn, 0086-755-26535295
Mr. Renqiang szurcs@sina.cn, 0086-755-26535295

THE GEORGE WASHINGTON UNIVERSITY

WASHINGTON, DC

Faculty Position in Developmental Neuroscience George Washington Institute for Neuroscience Department of Pharmacology and Physiology School of Medicine and Health Sciences

The George Washington Institute for Neuroscience is accepting applications for a tenure-eligible faculty member at the rank of Associate or Full Professor with expertise in developmental neuroscience. We seek an established investigator with an interest in the molecular, cellular or physiological mechanisms of mammalian neural circuit development and function in the cerebral cortex or other brain regions. The primary appointment will be in the Department of Pharmacology and Physiology in the School of Medicine and Health Sciences. This individual will also participate in medical and graduate education. The successful candidate will participate in collaborative research activities including development of multi-investigator projects for extramural funding. Salary and start-up funds will be commensurate with experience. The GW Institute for Neuroscience is a campus-wide research initiative with 45 faculty members in 7 Departments in the School of Medicine and Health Sciences, The Columbian College of Arts and Sciences, and our partner institution, Children's National Medical Center.

Basic Qualifications: Applicants must have a terminal degree (Ph.D. or M.D.) in an appropriate discipline and substantial accomplishments in biomedical research as demonstrated by a significant number of first and/or senior author publications in outstanding peer-reviewed journals as well as success in obtaining external research support. **To be considered,** please complete an online faculty application at <http://www.gwu.jobs/postings/18056> and submit a complete curriculum vitae, research and teaching statement, reprints of two recent publications, and the names of three individuals who can provide reference letters. Review of applications will begin on **November 22, 2013**, and will continue until the position is filled. Only complete applications will be considered.

GW is an Equal Opportunity/Affirmative Action Employer.



Assistant or Associate Professor Medical Oncology

The Dana-Farber Cancer Institute, Brigham and Women's Hospital and the Department of Medicine, Harvard Medical School, invite applications for a full-time appointment at the Assistant or Associate Professor level. This individual will develop a molecular target-based research program housed in the Department of Medical Oncology at the Dana-Farber Cancer Institute. The successful candidate should be committed to innovative cancer research in both the lab and clinic with the goal of advancing these discoveries toward novel therapeutic strategies. Applicants must have an MD, PhD or MD-PhD and a proven track record of outstanding research.

The candidate will work principally at the Dana-Farber Cancer Institute, an NCI-designated Comprehensive Cancer Center, and at the Brigham and Women's Hospital. Academic rank of Assistant/Associate Professor level at Harvard Medical School will be commensurate with experience, training and achievements.

Interested candidates must submit a curriculum vitae, a research plan and three letters of reference to: William Hahn, MD, PhD, Dana-Farber Cancer Institute, 450 Brookline Avenue, Boston, MA 02215. Please send submissions via email to: MCO_SEARCH@dfci.harvard.edu. Please direct telephone inquiries to Amy Dickson at 617-632-3155.



HARVARD
MEDICAL SCHOOL



BRIGHAM AND
WOMEN'S HOSPITAL

Dana-Farber Cancer Institute/Brigham and Women's Hospital/Harvard Medical School are Equal Opportunity/Affirmative Action Employers actively committed to increasing the diversity of our faculty; people with disabilities, veterans, women and members of underrepresented minority groups are therefore strongly encouraged to apply.



The mission of the National Renewable Energy Laboratory (NREL) is to develop renewable energy and energy efficiency technologies and practices, advance related science and engineering and transfer knowledge and innovations to address the nation's energy and environmental goals.

Center Director - Chemical and Materials Science Center Requisition #3155BR

Job/Research Summary

NREL is seeking a Center Director to lead its Chemical and Materials Science Center within the recently formed Materials and Chemical Science and Technology (MCST) Directorate. The Center's basic and applied research portfolio integrates theory, chemistry and materials science for understanding and developing renewable energy science and technologies including solar energy conversion for electricity and fuels, hydrogen storage and fuel cells, energy storage, and energy efficiency.

Knowledge and Experience

Extensive knowledge of renewable energy science and technology. Recognized publications and technical contributions in chemical or materials science along with national reputation. Program strategy, development and execution experience. Record of progressively complex assignments and applications, including Demonstrated success in management or technical leadership experience in a collaborative R&D environment.

EEO Policy / E-Verify

NREL's policy is to provide equal employment opportunities to all qualified persons without regard to race, age, color, sex, religion, national origin, marital or veteran status, or any other legally protected status.

To view the full description and to apply online, visit www.nrel.gov/employment and search for Job 3155.



Florida State University College of Medicine Tenure-track positions

The Department of Biomedical Sciences at the Florida State University College of Medicine invites applications for tenure track faculty positions at all ranks. Successful candidates will be expected to have (or develop) and sustain extramurally supported research and participate in medical and graduate education. Preference will be given to applicants with research in cancer biology or pharmacology with teaching experience in medical pharmacology or other major pre-clinical areas. Please submit a letter of application, curriculum vitae, an overview of future research plans, and a list of references in a single pdf file to biomedfacultysearch@med.fsu.edu. If interested please apply to Florida State University at <https://jobs.fsu.edu>, job ID #36246. We will begin to review the applications on **November 15, 2013**.

The College of Medicine has more than 150,000 sq. ft. of state-of-the-art laboratory space, core labs in proteomics, genomics, confocal microscopy, flow cytometry, and cell culture. Current research in the department is broadly focused on understanding the molecular bases of human diseases with focus areas including Neuroscience, Cancer Biology, and Cardiovascular Disease. See <http://med.fsu.edu/biomed> for more information.

The Florida State University is an Equal Opportunity/Affirmative Action Employer.

UNIVERSITY OF MICHIGAN Ecology or Evolutionary Ecology Faculty Position

The Department of Ecology and Evolutionary Biology at the University of Michigan seeks applicants for an assistant professor (tenure-track) position in ecology or evolutionary ecology. This is a university-year appointment with an expected start date of September 1, 2014. We seek applicants with a strong field component in her or his research program. We welcome applicants who work in any of the planet's major ecosystems but are especially interested in individuals who will leverage the facilities available at the University of Michigan, including world class biodiversity collections (e.g., birds, fishes, etc., at the Museum of Zoology), a local field research facility (the Edwin S. George Reserve), and a large educational and research facility in northern Michigan (the University of Michigan Biological Station). Museum curatorial activities may replace some teaching duties for appropriate candidates.

Applications should include a cover letter, CV, a concise statement describing your current and future plans for research, a statement of your teaching philosophy and experience, and evidence of teaching excellence (if any). Include names of three references. To apply, please see www.resources-eeb.lsa.umich.edu/eebsearch13/application.php. Review of applications will begin on **November 22, 2013** and continue until the position is filled.

Women and minorities are encouraged to apply and the University is supportive of the needs of dual career couples. The University of Michigan is an Equal Opportunity/Affirmative Action Employer.



Faculty Position in Physiological Sciences

The Department of Physiological Sciences at the Eastern Virginia Medical School (EVMS) is seeking candidates for a tenure track faculty position at the Assistant/Associate Professor level. Areas of strength in the Department include basic and translational research on fetal development and reproductive medicine, diabetes-obesity, ocular sciences, motor protein biochemistry and cardiovascular research. The successful applicant must have a Ph.D. and or M.D., postdoctoral training and an active externally funded research program. Competitive facilities, startup package, and core resources are available to support this position. The position will also involve participation in the teaching programs of the department to medical and health professions students and the Ph.D. Graduate Program in Biomedical Sciences. Women, military veterans and individuals from underrepresented minority groups are strongly encouraged to apply.

Interested applicants would need to apply at: <https://careers-evms.icims.com/jobs/>. Please include a letter of interest, an NIH biosketch and full curriculum vitae. For more information please contact **Dr. Diane Duffy**, duffydm@evms.edu, Search Committee Chair.

EVMS is an EOE/Affirmative Action Employer/M/F/D/V and Drug and Tobacco Free Workplace.



Bioengineering Faculty Position

The California Institute of Technology invites applications for a tenure-track assistant professor position in the Division of Biology and Biological Engineering. Bioengineering research at Caltech focuses on the application of engineering principles to the design, analysis, construction, observation and manipulation of biological systems, and on the discovery and application of new engineering principles inspired by the properties of biological systems.

Applications are invited in any area of bioengineering research. Candidates with strong commitments to research and teaching excellence are encouraged to apply. Initial appointments at the assistant professor level are for four years, and are contingent upon completion of the Ph.D. degree.

The Bioengineering Option (<http://www.be.caltech.edu>) includes faculty from the Divisions of Biology and Biological Engineering (<http://www.bbe.caltech.edu>), Chemistry and Chemical Engineering (<http://www.cce.caltech.edu>), Engineering and Applied Science (<http://www.eas.caltech.edu>), and Physics, Mathematics, and Astronomy (<http://www.pma.caltech.edu>).

Please submit online application at <https://applications.caltech.edu/job/bioengineering> and include a brief cover letter; curriculum vitae; relevant publications, a description of proposed research; and a statement of teaching interests. Instructions will be given for submission of letters of reference when you apply on-line.

Application review will commence immediately and continue until **December 1, 2013** or until the position is filled.

The California Institute of Technology is an Equal Opportunity/Affirmative Action Employer. Women, minorities, veterans, and disabled persons are encouraged to apply.



澳門大學
UNIVERSIDADE DE MACAU
UNIVERSITY OF MACAU

International Search for Academic Positions of Assistant Professor or above

The University of Macau is a leading higher educational institution in Macao and is making strides towards becoming internationally recognized for its excellence in teaching, research and service to the community. The University is growing rapidly with a number of new strategic initiatives including the relocation to a new campus and the establishment of the largest Residential College system in Asia. The new campus is 20 times larger than the present one with a projected fast growth of student intake and faculty size. English is the University's working language.

We plan to develop a strong team of top-notch scholars to help us realize our vision. Applications are therefore invited from those with excellent academic achievements in the following disciplines:

- * Business and Management
- * Education
- * Law
- * Liberal Arts and Humanities
- * Social Sciences
- * Mathematics, Sciences and Engineering
- * Health Sciences
- * Chinese Medicines

Remuneration and appointment rank offered will be competitive and commensurate with the successful applicants' academic qualification, current position and professional experience. The current local maximum income tax rate is 12% but is effectively around 5% - 7% after various discretionary exemptions.

For details about the above open positions and related information, please visit the following websites:

Job vacancy website: <http://www.umac.mo/vacancy>
University website: <http://www.umac.mo>
Macao government website: <http://www.gov.mo>

Further particulars about the job openings are available at <http://www.umac.mo/vacancy>. Kindly apply online through the E-application system. Applications will be accepted until the positions are filled. Applicants may consider their applications not successful if they were not invited for an interview within 3 months of application.

Human Resources Office
University of Macau, Av. Padre Tomás Pereira, Taipa, Macau
Website: <https://isw.umac.mo/recruitment>;
Email: senior.academic@umac.mo
Tel: +853 8397 8593 or +853 8397 8592;
Fax: +853 8397 8694

The effective position and salary index are subject to the Personnel Statute of the University of Macau in force.
The University of Macau reserves the right not to appoint a candidate. Applicants with less qualification and experience can be offered lower positions under special circumstances.

*****Personal data provided by applicants will be kept confidential and used for recruitment purpose only*****

*University of Macau -
An ideal place to pursue your career*

<http://www.umac.mo>

Woods Hole Oceanographic Institution



Fellowships for Postdoctoral Scholars

New or recent doctoral recipients with research interests associated with the following are encouraged to submit scholarship applications prior to January 5, 2014.

Departments - Awards related to the following areas are anticipated: Applied Ocean Physics & Engineering; Biology; Geology & Geophysics; Marine Chemistry & Geochemistry; Physical Oceanography

Institutes - Each Institute fosters interdisciplinary research addressing critical issues, and will award a scholarship to support related research: Ocean and Climate Change Institute; Coastal Ocean Institute; Deep Ocean Exploration Institute; Ocean Life Institute.

The Cooperative Institute for the North Atlantic Region will award a Fellowship in one of five theme areas: Ecosystem Forecasting, Ecosystem Monitoring, Ecosystem Management, Protection and Restoration of Resources, Sustained Ocean Observations and Climate Research.

Awards are competitive, with primary emphasis placed on research promise. Scholarships are 18-months with an annual stipend of \$57,500, a research budget and eligibility for health and dental insurance.

Recipients are encouraged to pursue their own research interest in association with resident staff. Communication with potential WHOI advisors prior to submitting an application is encouraged.

Further info may be obtained at:
<http://www.whoi.edu/postdoctoral>

An Equal Opportunity/Affirmative Action Employer



TEXAS TECH UNIVERSITY

Structural Biology: Biological NMR Spectroscopy

The Department of Chemistry and Biochemistry invites applications for a faculty member, at the rank of Assistant Professor (tenure-track), in the area of **Biological NMR Spectroscopy**. We are especially interested in applicants whose research programs will complement existing areas of strength at TTU, such as structure, function and dynamics of membrane proteins, biomolecules involved in cancer, and plant proteins. However, all qualified candidates are encouraged to apply. A Ph.D. and postdoctoral experience are required. Evidence of the ability to generate a well-funded research program and a demonstrated commitment to excellence in teaching and service are essential. The successful candidate for this position will be part of the major new **Structural Biology** initiative at TTU which was launched last year. One faculty member for this initiative has been hired and a 600 MHz NMR spectrometer will be installed in early 2014. The hiring of additional faculty in this area and the acquisition of additional instrumentation is part of this initiative. The Department of Chemistry and Biochemistry is among the top academic units at Texas Tech University in terms of research funding, publications and graduate education. Texas Tech University is classified as a doctoral research-extensive university by the Carnegie Foundation; it has an enrollment of more than 33,000 students, and is one of the major, state-supported, multidisciplinary universities of the Southwest. A School of Medicine is located on the main campus in Lubbock.

All applications must be submitted online. Online faculty application for **requisition number 89872** can be found at <http://jobs.texasstate.edu/postings/57603>. Applications must include a curriculum vitae, statement of proposed research and teaching philosophy. Applicants must also arrange to have three confidential letters of recommendation sent on their behalf to the chair of the search committee, **Dr. Joachim Weber, Department of Chemistry and Biochemistry, Texas Tech University, Box 41061, Lubbock, TX 79409-1061 (joachim.weber@ttu.edu)**. Evaluation of applications will start on **November 15, 2013**, and continue until the position is filled.

Texas Tech University is an Affirmative Action/Equal Opportunity Employer, committed to excellence through diversity. Texas Tech welcomes applications from minorities, women, veterans, persons with disabilities, dual-career couples, and all qualified persons.

THE GEORGE WASHINGTON UNIVERSITY

WASHINGTON DC

The Department of Microbiology, Immunology and Tropical Medicine, and the newly established Research Center for Neglected Diseases at The George Washington University's School of Medicine and Health Sciences, seeks new faculty members to establish vibrant, extramurally funded research programs.

Tenure-track faculty positions at the assistant professor level are available in these thematic areas: • HIV immunobiology, especially related to HIV "functional cure" or HIV vaccine research. • Immunology, genetics and pathogenesis of infection-related cancers including those caused by helminths (e.g. flukes, protozoans) and viruses (e.g., EBV, HTLV, and HHV8). • Neglected tropical disease pathogens and vector biology. • Human immunology, especially innate immune mechanisms, vaccines, and mucosal immunology/human microbiome.

Successful applicants will be expected to establish or expand an extramurally funded and internationally recognized research program; experience or interest in basic and translational research on infectious diseases in endemic areas are of special interest.

Basic qualifications: Applicants must have a PhD and/or MD/DVM or equivalent degree, in related relevant disciplines (HIV immunobiology, Immunology, Microbiology, Parasitology); postdoctoral and/or other research experience; a compelling publication track record; grant writing experience; demonstrated interest in teaching graduate and health care students; and evidence of potential to attract extramural funding.

Highly competitive salary, start-up funds, and space within the new NIH-funded collaborative laboratory space are available. Application procedure: Applications should be completed on-line at <http://www.gwu.jobs/postings/18484> and should include a cover letter emphasizing specific qualifications, curriculum vitae, a 3-5 page research plan, and names and contact information for three referees. Only complete applications will be considered. Application review will begin **November 30th 2013** and will continue until the positions are filled. For further information about the positions please contact **Dr. Douglas F. Nixon**, Department Chair, The George Washington University at mitmfacs@gwu.edu

The George Washington University is an Equal Opportunity/Affirmative Action Employer and seeks to attract active, culturally and academically diverse faculty of the highest caliber.



UNIVERSITY AT ALBANY
State University of New York

Junior and Senior Faculty Positions at The RNA Institute

RNA ENVIRONMENTAL BIOCHEMIST (tenured/tenure track): RNA epigenetics, environmental and drug insults to RNA with functional consequences. For full position description and application:

<http://albany.interviewexchange.com/jobofferdetails.jsp?JOBID=43450>

RNA CANCER BIOLOGIST (tenure track): Role of RNA in oncogenesis or cancer therapeutics, or a RNA based genomics approach to cancer biology. For full position description and application:

<http://albany.interviewexchange.com/jobofferdetails.jsp?JOBID=42800>

LIFE SCIENCE ENTREPRENEURSHIP & TECHNOLOGY COMMERCIALIZATION (tenured/tenure track): Research, technology advancement and commercialization, and instruction that elevates and expands UAlbany's program offerings to science and business students in one of the most important and dynamic areas of growth in the US. For full position description and application:

<http://albany.interviewexchange.com/jobofferdetails.jsp?JOBID=43452>

Successful candidates will be members of The RNA Institute (<http://www.albany.edu/rna>) with state-of-the-art laboratories in recently completed Institute facilities residing in the modern Life Sciences Research Building (<http://www.albany.edu/lifesciences>). The Institute comprises more than 35 University and regional research labs and institutions including the Wadsworth Center, Rensselaer Polytechnic Institute, and Albany Medical College, and some 20 other prominent investigators nationwide. This creates an outstanding environment for interdisciplinary research collaborations.

Successful candidates will be offered competitive salaries, start-up packages and research space that is consistent with their rank and area of research.

Submit applications on-line to the appropriate website above. Include CV, publications and funding, descriptions of research accomplishments and experience in RNA science, future research plans, and teaching interests. Applications must address their ability to work with and instruct a culturally diverse population. Applicants must arrange for three or more reference letters sent on their behalf directly to Paul F. Agris, Ph.D., rna@albany.edu.

The University at Albany is an EEO/AA/IRCA/ADA employer.



THE UNIVERSITY OF CHICAGO

Academic Career Opportunities

Microbiology Faculty Position Req #01884

The University of Chicago's Department of Microbiology invites applications for a tenure-track faculty position at the rank of Assistant Professor, although, depending on qualifications, candidates may be proposed for a senior appointment. Applicants must have a MD, PhD, or MD/PhD and relevant postdoctoral training. The successful candidate is expected to develop an extramurally-supported research program focusing on host-pathogen interactions of viral, parasitic, fungal or bacterial infectious agents. Candidates are also expected to contribute to departmental teaching.

The University of Chicago maintains extensive core facilities in support of microbiological research including facilities for experiments with gnotobiotic animals and risk group 1-3 infectious agents. A competitive salary and start-up package will be provided. Review of applications will begin on **January 1, 2014** and continue until the position is filled. Interested applicants must submit a cover letter, curriculum vitae, the names and contact information for at least three references, and a statement of research interests emphasizing career goals at the following url: <http://tinyurl.com/mdh9fpj>.

The University of Chicago is an Affirmative Action / Equal Opportunity Employer.

<http://tinyurl.com/mdh9fpj>



EPPLEY INSTITUTE
FOR RESEARCH IN CANCER



The Fred & Pamela Buffett Cancer Center, a National Cancer Institute-designated Cancer Center at the University of Nebraska Medical Center, seeks outstanding candidates for the position of Cancer Center Co-Program Leader for the Cancer Genes and Molecular Regulation (CGMR) Program. The Co-Program Leader position will include an appointment in the Eppley Institute for Research in Cancer with academic rank commensurate with experience.

The Fred & Pamela Buffett Cancer Center is in a dynamic growth phase and committed to expansion of all its research programs. Currently the University of Nebraska Medical Center and its hospital partner, The Nebraska Medical Center, is building a \$323 million cancer center complex on the main campus. The Fred & Pamela Buffett Cancer Center will include a 10-story, 260,000-square-foot cancer research tower, a multidisciplinary outpatient clinic (surgical, medical, and radiation oncology), infusion center, radiation treatment facility, and a 108-bed inpatient cancer hospital. This is the largest capital project in the history of the University of Nebraska. In addition, the University is also conducting a \$100 Million capital campaign for program development in the Fred & Pamela Buffett Cancer Center.

The successful applicant will have joint responsibility for the overall direction and development of the Fred & Pamela Buffett Cancer Center's CGMR program. Responsibilities also include maintaining an independent research program and fostering the continued development of basic research programs and interdisciplinary collaborations. This person will advise the Fred & Pamela Buffett Cancer Center Director on promising areas of research and provide direction to CGMR program members in pursuing research objectives.

Individuals with projects focused on (a) identifying novel therapeutic agents using high throughput/ systems biology, (b) targeted cancer therapies, and/or (c) experimental cancer models are encouraged to apply.

The anticipated start date is July 1, 2014. EEO/AA individuals from diverse backgrounds are encouraged to apply. To apply, go to <https://jobs.unmc.edu> and reference requisition #2013-183. Additional information at <http://www.unmc.edu/cancercenter>.



California NanoSystems Institute (CNSI)
at the
University of California Santa Barbara
is pleased to announce
the 2013-14 competition for the

ELINGS PRIZE FELLOWSHIPS in EXPERIMENTAL SCIENCE

The Elings Prize postdoctoral fellowships provide a salary of \$60,000/year for two years, potentially renewable for a third, with benefits and research funds, for up to 3 fellows. Eligible research can be in any area of the physical sciences, biology, or engineering. Successful applicants will work with, and receive partial support from, experimentalists on the CNSI faculty; **applications must be coordinated with the relevant faculty supervisor(s)**. For more information, visit http://www.cnsi.ucsb.edu/admin/funding/elings_fellowships/.

Applicants should submit a cover letter; CV; a one-page research proposal; and arrange for 3 supporting letters, all submitted via the website <http://fellow.cnsi.ucsb.edu/>.

The deadline for applications is
January 31, 2014.

The University of California is an Equal Opportunity/Affirmative Action Employer.



Faculty Positions in Viral Immunopathogenesis at the Vaccine & Gene Therapy Institute of Florida

The Vaccine & Gene Therapy Institute of Florida (VGTI Florida) is seeking to recruit outstanding immunologists/virologists to direct research programs in basic and translational human immunology. Priority areas of research include immunopathogenesis, emerging viral pathogens including influenza and flaviviruses, vaccine development, adjuvants, and inflammation. We are seeking scientific leaders in the areas of molecular virology, immune response to infection and antiviral/vaccine strategies. Priority will be given initially to established investigators with vigorous research programs investigating HIV, influenza, Dengue and emerging viruses. VGTI Florida is one of the internationally recognized research institutes invited to locate to Florida as part of a State-sponsored initiative to enhance biomedical research. Research at VGTI Florida focuses on human innate and adaptive immune responses to infectious diseases and cancer.

Research themes at VGTI Florida include:

- HIV-1 and emerging viral pathogens
- Vaccine development and adjuvants
- Cancer Immunology and immunotherapy
- Inflammation and diseases of aging

VGTI Florida occupies a new 100,000 sq. ft. state-of-the-art facility in Port St. Lucie, FL, located on the sunny Atlantic coast in a Biotech corridor just north of Palm Beach. Successful candidates (PhD and/or MD) will have an established extramurally-funded research program and a strong publication record in one of the priority areas described above. The positions have highly competitive salary and startup packages, with access to cutting edge Genomics, Bioinformatics and Flow Cytometry core facilities as well as BSL3/ABSL3 containment facilities within the Institute. For more information, including a description of the Faculty and their research interests, please visit: www.vgtifl.org. Qualified candidates should submit their curriculum vitae, a 2-page description of their proposed research program and the names/contact information of three references to:

Jill Hackett
Executive Director, Human Resources
Vaccine & Gene Therapy Institute of Florida

Applications must be sent via email to: search@vgtifl.org. Review of applications will commence immediately and continue until the positions are filled.

VGTI Florida is an Equal Opportunity Institution committed to recruiting, hiring, and promoting qualified minorities, women, individuals with disabilities, and veterans.



Plant Metabolic Systems Assistant Professor

The School of Integrative Biology and the Department of Plant Biology at the University of Illinois, Urbana-Champaign seek an outstanding plant biochemist/systems biologist who studies primary or secondary plant metabolic pathways and networks through the use of cutting-edge measurement and/or modeling techniques. Research areas of interest in plant metabolism include, but are not limited to: flux analysis; improvement of productivity/quality of food and fuel crops; biotic or abiotic interactions; metabolic engineering.

The successful candidate will be expected to develop an externally funded research program, teach at undergraduate and graduate levels, and collaborate with faculty to develop research and education initiatives in plant biochemistry and metabolic systems. A Ph.D. or equivalent in a relevant field is required. Postdoctoral experience is highly desirable.

The University of Illinois provides a highly collaborative and supportive academic environment for cross-disciplinary plant systems research given its strengths in photosynthesis, global change, molecular and cellular biology, genomics, ecology, chemistry and computation. Relevant interacting units include the: Institute for Genomic Biology; Energy Biosciences Institute; National Center for Supercomputer Applications; Bill and Melinda Gates Foundation funded Realizing Improved Photosynthetic Efficiency (RIPE) project; Departments of Biochemistry, Microbiology, Bioengineering, Chemical and Biomolecular Engineering, and Computer Science.

The appointment is for a full-time, nine-month, tenure-track **Assistant Professor**. Target start date is 16 August 2014. Salary is commensurate with experience. To ensure full consideration, please create your candidate profile through <http://go.illinois.edu/PlantMetabolic> and upload your application letter, curriculum vitae, summary of research and plans, teaching philosophy and experience, and contact information including e-mail addresses for three professional references by **1 December 2013**. After a review of the candidate's record, the search committee may then contact the applicant about soliciting letters of reference. Applicants may be interviewed before the closing date; however, no hiring decision will be made until after that date. For further information contact Plant Metabolic Systems Search Chair, sib@life.illinois.edu.

*Illinois is an Affirmative Action /Equal Opportunity Employer and welcomes individuals with diverse backgrounds, experiences, and ideas who embrace and value diversity and inclusivity.
(www.inclusiveillinois.illinois.edu).*



Assistant Professor Department of Biochemistry and Redox Biology Center

The Department of Biochemistry and the Redox Biology Center (RBC) at UNL, invite applications for a **tenure-leading Assistant Professor position in biophysical chemistry**. This position is a 9-month appointment and is part of the strategic plans of UNL, the Institute of Agriculture and Natural Resources (IANR), and Department of Biochemistry/RBC to increase research capacity in the areas of redox and stress biology that impact human health. Applicants with programs centered on biophysical approaches to address important questions in redox biology are desirable. The RBC, supported as a Center of Biomedical Research Excellence by the National Institutes of Health, is an emphasis group for research in redox biology involving investigators at UNL, University of Nebraska Medical Center, and Creighton University in Omaha. Redox regulation and thiol-based redox signaling, biochemistry of redox-active trace elements, structural biology, redox proteomics/metabolomics, and mitochondrial dynamics are some of the current areas of investigation in the Center.

A Ph.D. degree (or equivalent) in a related scientific field and at least 1 year of postdoctoral experience (or equivalent) are required. It is expected that the investigator will establish a nationally recognized and externally supported research program and contribute to the undergraduate and graduate education missions of the Department of Biochemistry. The position is housed in the George W. Beadle Center, which includes state-of-the-art teaching and research laboratories, and research core facilities. The RBC and the Department of Biochemistry maintain a wide array of instrumentation for biophysical chemistry research and operate core research facilities in the areas of metabolomics/proteomics, biophysical spectroscopy, and X-ray crystallography. The city of Lincoln boasts an outstanding quality of life that includes fine culinary and artistic treasures, a budding live music scene and numerous parks, golf courses and bike trails. Forbes ranked Lincoln as the 7th best city in the US for business and careers in 2012.

To learn more about UNL, the Biochemistry Department, and the RBC visit <http://unl.edu>. Applicants should go to <http://employment.unl.edu>, search for requisition number F_130209, Click on "Apply to this job", complete the form, and attach a letter of application, curriculum vitae, teaching statement, and brief description (2-3 pages) of research program and goals. Applicants must arrange for 3 confidential letters of reference to be sent directly to: **Biophysical Chemistry Search Committee, Department of Biochemistry, University of Nebraska, N200 Beadle Center, Lincoln, NE 68588-0664, USA**, or by e-mail to biochemistrysearch@unl.edu. Review of applications will begin on **December 2, 2013** and continue until the position is filled. The start date for this position is August 15, 2014.

The University of Nebraska has an active National Science Foundation ADVANCE gender equity program, and is committed to a pluralistic campus community through affirmative action, equal opportunity, work-life balance, and dual careers.

POSITIONS OPEN

FORENSIC SCIENCE

The Chemistry Department and the National Center for Forensic Science at the University of Central Florida (UCF) anticipate hiring two nine-month, tenure-track **ASSISTANT, ASSOCIATE, or FULL PROFESSORS** in forensic science beginning August 2014.

Minimum qualifications at the Assistant Professor level are a Ph.D. in chemistry or appropriately related field from an accredited institution, at least one year of postdoctoral research, and a demonstrated track record of research productivity in forensic science. Candidates for appointment as Associate or Full Professor will exhibit a track record of competitive funding for forensic science research.

Specialization in forensic toxicology is of specific interest; however, applications in all areas of forensic science are welcome.

Candidates hired at the Assistant Professor level are expected to develop externally funded, nationally competitive research programs. State-of-the-art laboratory space and a competitive startup package can be expected. Successful candidates at all levels will contribute to teaching in both the undergraduate and graduate programs. The Chemistry Department at UCF offers B.S. Chemistry, B.S. Forensic Science, M.S. Chemistry, M.S. Forensic Science, and Ph.D. Chemistry degrees. The National Center for Forensic Science at UCF is an internationally recognized leader in forensic science research. The University of Central Florida, located in Orlando, FL, is the nation's second largest university with nearly 60,000 students and is continuing to build internationally recognized research programs.

Applicants must apply online at **website: <http://www.jobswithucf.com>** and submit letter of application, curriculum vitae, description of research plans, teaching philosophy, and interests, and names of at least three references. Please arrange to have three letters of reference sent to **e-mail: chemstaff@ucf.edu**. Please indicate in the subject line "Forensic Toxicology or Forensic Other." Review of applications will begin December 1, 2013 and continue until the position is filled. *The University of Central Florida is an Equal Opportunity/Equal Access/Affirmative Action Employer.*

TENURE-TRACK ASSISTANT/ASSOCIATE PROFESSORSHIP in Cardiology, Medical University of South Carolina

The Gazes Cardiac Research Institute in the Cardiology Division of the Department of Medicine at the Medical University of South Carolina (MUSC) is seeking applicants for a tenured-track faculty position of Assistant/Associate Professor. A competitive laboratory start up package will be provided to the successful candidate to support the development of an independent, funded research program in cardiovascular biology. Preference will be given to candidates whose research investigates some aspect of the mechanisms mediating complex cardiac arrhythmias and sudden death. The successful candidate will be expected to integrate their research program into the highly collaborative and supportive existing research program. Applicants for associate professor must have an active, established extramurally funded research program with a record of peer reviewed publications and national/international recognition. Candidates must have a Ph.D. or M.D. in a related field and rigorous postdoctoral experience leading to publications in top tier journals. The Medical University of South Carolina offers a highly interactive-environment, a strong infrastructure for research and is located in the historic city of Charleston. Interested individuals should submit a letter of interest, curriculum vitae, a detailed statement of research plans, and the names and contact information of three references via the MUSC employment **website: <https://www.jobs.musc.edu>** **requisition ID 048268**. Inquiries regarding this position can be sent to **Donald Menick, Ph.D., Director, Gazes Cardiac Research Institute via e-mail: menickd@musc.edu**.

MUSC is an Equal Opportunity Employer, promoting workplace diversity.

Get your questions answered.

Careers Forum

www.ScienceCareers.org

Department of Electrical and Systems Engineering



**Penn
Engineering**

Tenured/Tenure-Track Faculty Position

The Department of Electrical and Systems Engineering of the School of Engineering and Applied Science at the **University of Pennsylvania** invites applications for tenured and tenure-track faculty positions at all levels. Candidates must hold a Ph.D. in Electrical Engineering, Systems Engineering, or related area. The department seeks individuals with exceptional promise for, or proven record of, research achievement, who will take a position of international leadership in defining their field of study, and excel in undergraduate and graduate education. Leadership in cross-disciplinary and multi-disciplinary collaborations is of particular interest. We are interested in candidates in all areas that enhance our research strengths in

1. **Nanodevices and nanosystems** (nanophotonics, nanoelectronics, integrated devices and systems at nanoscale),
2. **Circuits and computer engineering** (analog and digital circuits, emerging circuit design, computer engineering, embedded systems), and
3. **Information and decision systems** (communications, control, signal processing, network science, markets and social systems).

Prospective candidates in all areas are strongly encouraged to address large scale societal problems in energy, transportation, health, economic and financial networks, critical infrastructure, and national security. Diversity candidates are strongly encouraged to apply. Interested persons should submit an online application at <http://facultysearches.provost.upenn.edu/postings/40> including curriculum vitae, statement of research and teaching interests, and the names of at least four references. Review of applications will begin on **December 1, 2013**.

The University of Pennsylvania is an Equal Opportunity Employer. Minorities/ Women/Individuals with Disabilities/Veterans are encouraged to apply.



VirginiaTech
Invent the Future

TENURE-TRACK ASSISTANT PROFESSOR: CHRONIC HUMAN DISEASES

The Department of Biochemistry at Virginia Tech seeks applicants for a tenure track assistant professor position working in the area of chronic human diseases. We seek candidates who apply contemporary metabolic, proteomic, genomic, or systems biology approaches to addressing the causes, treatment and/or comorbidities of diseases such as – but not limited to — hypertension, diabetes, obesity, heart disease, Alzheimer's disease, osteoporosis, or cancer. The successful candidate will be part of a multi-department cluster hire organized around the theme of Food Systems and Health that represents part of an ongoing program to expand Virginia Tech's portfolio in the area of biomedical research. It is expected that the successful candidate will build and sustain an internationally-recognized, extramurally-funded research program, participate in student instruction, and will be open to opportunities to engage in collaborative research. Competitive start-up packages will be provided. In the past several years Virginia Tech has made major investments in their biomedical research infrastructure that include the establishment of a comprehensive biological mass spectrometry laboratory, a new vivarium, new BSL-3 laboratories, etc. The Department of Biochemistry (<http://www.biochem.vt.edu>) is currently home to sixteen tenure and tenure-track faculty working in areas ranging from molecular dynamics to infectious disease, metabolomics, drug development, signal transduction, and molecular microbiology.

Review of applications will begin on **November 11, 2013** and continue until the position is filled. Applicants must apply online (<https://listings.jobs.vt.edu>, use posting number **TR0130115**). When applying for this position, please attach a cover letter, curriculum vitae, list of three references, statement of future research plans, and description of teaching philosophy to the online application; and arrange to have three letters of reference sent electronically to **Prof. Jinsong Zhu** (zhujin@vt.edu).

*Virginia Polytechnic Institute and State University is an
Equal Opportunity Educator and Employer.*



Join the Conversation!

Twitter is a great way to connect with AAAS members and staff about the issues that matter to you most. Be a part of the discussion while staying up-to-date on the latest news and information about your personal member benefits.

**Follow us @AAASmember
and join the conversation
with #AAAS**



MemberCentral.aaas.org

There's only one GALILEO GALILEI

Born in 1564, Galileo Galilei once contemplated a career in the priesthood. It's perhaps fortunate for science that upon the urging of his father, he instead decided to enroll at the University of Pisa. His career in science began with medicine and from there he subsequently went on to become a philosopher, physicist, mathematician, and astronomer, for which he is perhaps best known. His astronomical observations and subsequent improvements to telescopes built his reputation as a leading scientist of his time, but also led him to probe subject matter counter to prevailing dogma. His expressed views on the Earth's movement around the sun caused him to be declared suspect of heresy, which for some time led to a ban on the reprinting of his works.

Galileo's career changed science for all of us and he was without doubt a leading light in the scientific revolution, which is perhaps why Albert Einstein called him the father of modern science.

Want to challenge the status quo and make the Earth move? At *Science* we are here to help you in your own scientific career with expert career advice, forums, job postings, and more — all for free. For your career in science, there's only one *Science*. Visit ScienceCareers.org today.



For your career in science, there's only one **Science**



ScienceCareers.org